Metabolic Syndrome:

Definition and prevalence in Portugal and associations with hypovitaminosis D, thyroid dysfunction and autoimmunity.

Porto | 2018

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Dissertation of the candidature for the degree of Doctor presented to the Faculty of Medicine of the University of Porto

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List of original publications

Under Article 8 of Decree-Law no. 388/70, the following publications are part of this dissertation:

I. Raposo L, Severo M, Santos AC. Adiposity cut-off points for cardiovascular disease and diabetes risk in the Portuguese population: The PORMETS study. PLoS ONE 2018;13(1): e0191641. doi: 10.1371/journal.pone.0191641.

II. Raposo L, Severo M, Barros H, Santos

AC. The prevalence of the metabolic syndrome in Portugal: the PORMETS study. BMC Public Health 2017;17:555. doi: 10.1186/s12889-017-4471-9.

III. Raposo L, Martins S, Ferreira D, Guimarães JT, Santos AC. Vitamin D, parathyroid hormone and metabolic syndrome – the PORMETS study. BMC Endocr Disord 2017;17(1):71. doi: 10.1186/s12902-017-0221-3.

IV. Raposo L, Martins S, Ferreira D, Guimarães JT, Santos AC. Metabolic Syndrome, Thyroid Function and Autoimmunity – The PORMETS Study. Submitted to BioMed Research International.

Throughout my PhD, I was involved in the design, implementation and monitoring of the data collection of the PORMETS Study, which served as a basis for the elaboration of the articles that integrate this thesis. I was responsible, along with my doctoral supervisor, for the definition of the hypotheses under study. I performed the statistical analysis and interpretation of the results, drafted the initial version of the manuscripts and prepared their final versions for submission to scientific journals.

This research was carried out at the Institute of Public Health of the University of Porto, under the guidance of Professor Ana Cristina Santos (Faculty of Medicine and Institute of Public Health of the University of Porto) and the co-supervision of Professor João Tiago Guimarães (Faculty of Medicine and Institute of Public Health of the University of Porto).

The PORMETS study, which served as the basis for this thesis, was funded by the Insulin-Resistance Study Group and Thyroid Study Group of the Portuguese Society of Endocrinology, Diabetes and Metabolism and by the Bayer Healthcare Laboratory, with the support of all Regional Health Administrations of Continental Portugal and the Institute of Public Health of the University of Porto.

Acknowledgements

To the participants and collaborators of the PORMETS study, without which this thesis would not have been possible.

To the members of the Insulin-Resistance Study Group of the Portuguese Society of Endocrinology, Diabetes and Metabolism, where this study was born.

To Professor Henrique de Barros and the Institute of Public Health of the University of Porto, to whom I presented the project and who gave me the conditions to do it.

To Professor Ana Cristina Santos, for her help in all phases of the study and for her crucial role in guiding my thesis.

To Professor Tiago Guimarães, for his help in the laboratory study and the co-orientation of my thesis.

To my Professors of the Public Health Institute of the University of Porto, for their contribution to my training in Public Health.

To the Faculty of Medicine and to the Institute of Public Health of the University of Porto for receiving me so well.

To the Board Administration of the *Centro Hospitalar de Lisboa Ocidental* and to my Director, Dr. Carlos Vasconcelos, for their understanding regarding this personal project and for the authorization granted to be able to perform it.

To my parents, Joaquim Marques Raposo and Graciosa Gomes Duarte Raposo, to whom I owe my origins and all that I am.

To Cláudia Freitas, my life companion, without whom it would not have been possible to carry out this mission.

To my youngest son, Vasco Raposo, who pulled me to Porto.

To my daughters, Rita Raposo and Marta Raposo, for all the understanding and support.

To Diogo Horta, a friend always present.

To all the friends and many others not mentioned, who contributed to completing this project.

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Abbreviations

AACE	American Association of Clinical Endocrinologists
ACE	American College of Endocrinology
ADA	American Diabetes Association
AHA	American Heart Association
AIT	Chronic autoimmune thyroiditis
AMPK	AMP-activated protein kinase
AO	Abdominal obesity
ATGL	Adipose tissue triglyceride lipase
ATP III	Adult Treatment Panel III
BAI	Body adiposity index
BMI	Body mass index
BP	Blood pressure
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
ChREBP	Carbohydrate response element binding protein
CI	Confidence interval
CNPD	Comissão Nacional de Proteção de dados
CVD	Cardiovascular disease
DAG	Diacylglycerol

EGIR	European Group for Study of Insulin Resistance
FFA	Free fatty acids
FT3	Free triiodothyronine
FT4	Free thyroxine
GLUT4	Glucose transporter protein 4
HC	Hip cicumference
HDL	High-density lipoprotein
HOMA	Homeostatic model assessment
HR	Hazard ratio
hs-CRP	High sensitivity C-reactive protein
Hsl	Hormone-sensitive lipase
IAS	International Atherosclerosis Society
IASO	International Association for the Study of Obesity
IDF	International Diabetes Federation
IFG	Impaired fasting glycaemia
IGT	Impaired glucose tolerance
IL-6	Interleukin 6
IOM	Institute of Medicine
IR	Insulin resistance
IRS	Insulin resistance syndrome

JIS	Join interim statement
LPL	Lipoprotein lipase
LR	Likelihood ratio
MetS	Metabolic syndrome
miRNA	Micro ribonucleic acid
NCEP	National Cholesterol Education Program
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart, Lung, and Blood Institute
NPV	Negative predictive value
NUTS	Nomenclature of territorial units for statistics
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor-1
PKC	Protein Kinase C
PI3K	Phosphatidylinositol 3-kinase
PTH	Parathyroid hormone
RAAS	Renin-angiotensin-aldosterone system
ROC	Receiver operating characteristic
RR	Relative risk
SAA3	Serum amyloid A 3
SAT	Subcutaneous adipose tissue

SCH	Subclinical hypothyroidism
SD	Standard deviation
SHyper	Subclinical hyperthyroidism
SNS	Sympathetic nervous system
SREBP1c	Sterol regulatory element binding protein 1c
T3	Triiodothyronine
T4	Thyroxine
TgAb	Thyroglobulin antibodies
TNF-α	Tumor necrosis factor alpha
TPOAb	Thyroid peroxidase antibodies
TSH	Thyroid-stimulating hormone
UACR	Urinary albumin-to-creatinine ratio
UAE	Urinary albumin excretion
USA	United States of America
UV	Ultraviolet
VAT	Visceral adipose tissue
VDR	Vitamin D receptor
VLDL	Very low-density lipoprotein
VitD	Vitamin D
VITD2	Ergocalciferol

VitD3	Cholecalciferol
25(OH)D	25-hydroxyvitamin D
1α,25(OH)^{2}D	1alpha, 25-dihydroxyvitamin
DWC	Waist circumference
WHF	World Heart Federation
WHO	World Health Organization
WHR	Waist-to-hip ratio
WHtR	Waist-to-height ratio

Abstract

Metabolic syndrome is a clinical entity that integrates a cluster of risk factors for cardiovascular disease and type 2 diabetes and shares common pathophysiological mechanisms among which stand out insulin resistance and obesity.

The inclusion of an adipose measure in the most recent definitions of the metabolic syndrome was justified by the contribution of adiposity to the risk of cardiovascular disease and type 2 diabetes as well as to the pathophysiology of the syndrome. Adipose tissue may contribute to the syndrome through mechanisms related to insulin resistance and inflammation. Measures of abdominal obesity, that best translate visceral adipose tissue, may be better indicators of the risk associated with adiposity. Among these measures of abdominal obesity, the waist circumference has been highlighted, being used in the most recent definitions of the metabolic syndrome. Several cut-off points have been proposed for the definition of the waist circumference component of the metabolic syndrome. According to the most recent definition of the metabolic syndrome, two alternative cut-off points have been proposed for European populations ("European" and "Europid"). These two sets of waist circumference cut-off points, estimated in Caucasoid population, would be the ones that best identified body mass index values equal to or greater than 25 kg/m^2 (80 cm in women and 94 cm in men) and 30 kg/m^2 (88 cm women and 102 cm in men). These two different cut-off points were also adopted by World Health Organization for the stratification of the metabolic risk associated with abdominal obesity (high risk and very high risk, respectively for lower and higher cut-off points). However, in addition to the more

recent recommendations on the metabolic syndrome, suggesting the use of customized waist circumference cut-off points for specific populations, a growing number of studies from different countries and ethnic groups have estimated their own cut-off points, that better identify the risk associated with the metabolic syndrome. In Portugal, the prevalence of abdominal obesity defined according to the cut-off points proposed by the World Health Organization is quite high, especially in women. Taking into account the numbers on the national prevalence of abdominal obesity, the suitability for the Portuguese population of the cut-off points for the waist circumference proposed by the most recent definition of the metabolic syndrome can be questioned. However, no study on the subject has yet been conducted in Portugal. In addition, several studies have suggested that other measures of abdominal obesity, such as waist-to-height ratio, may be more adequate than the waist circumference to estimate the risk associated with the metabolic syndrome.

The metabolic syndrome has a high prevalence in Portugal, when compared with most European countries, United States of America and other regions of the globe. According to several cross-sectional studies, the national prevalence of the metabolic syndrome ranged from 24% to 28.4%, 42% to 66% and 37.2% to 69.4%, according to the Adult Treatment Panel III, International Diabetes Federation and Joint Interim Statement definitions, respectively. The high national prevalence of obesity, type 2 diabetes and hypertension may have contributed to these numbers, along with lifestyle-related behaviours and socio-economic characteristics.

Several studies around the world have found differences in the prevalence of the metabolic syndrome among rural and urban populations. However, none of the studies

conducted so far in Portugal have addressed the differences between rural and urban populations.

The endocrine system plays an important role in the regulation of energy balance, blood pressure and insulin sensitivity as well as glucose and lipid metabolism. Several endocrine diseases have been associated with the metabolic syndrome and the risk of cardiovascular disease and type 2 diabetes. Among them, hypovitaminosis D and thyroid dysfunction and autoimmunity stand out for their timeliness and prevalence. In Portugal, with the exception of studies on the prevalence of hypovitaminosis D carried out in specific groups, hypovitaminosis D and thyroid dysfunction and autoimmunity have been poorly studied, particularly in relation to cardiovascular and type 2 diabetes risk.

The main objective of this thesis was to study the metabolic syndrome and its determinants in Portugal with a special focus on the influence of obesity and frequent endocrine pathologies, including hypovitaminosis D and thyroid dysfunction and autoimmunity. To achieve this objective, a number of specific tasks have been set. Firstly, the adiposity measures that best estimated the risk associated with the metabolic syndrome, as well as their respective cut-off points, were identified in a sample of the Portuguese adult population (Paper I). Secondly, the prevalence of the metabolic syndrome and its determinants was analysed in the same population, taking into account the cut-off points identified in the previous step and the distribution of the population by districts, NUTS II and urban or non-urban residence (Paper II).

Subsequently, the prevalence of hypovitaminosis D, as well as the determinants of serum levels of 25-hydroxy vitamin D and parathyroid hormone, and its possible

associations with the metabolic syndrome and its components were analyzed in a sub-sample of the same population. (Paper III). Finally, the prevalence of thyroid dysfunction and thyroid antibody positivity were analyzed in the same sub-sample and the associations of thyroid-stimulating hormone, thyroid hormones and thyroid antibodies with the metabolic syndrome and its components were studied (Paper IV).

To achieve our objectives, a sample of adults enrolled in primary health care centers (PORMETS study) was used. In each of the 18 mainland Portuguese districts two health care centers were included, one of the capital and another representative of the non-urban area. In each health care center, 120 participants were randomly selected from the general practitioner´s patient lists. A total of 4095 participants were included between February 2007 and July 2009. A subsample including 500 participants, randomly selected from the initial PORMETS sample, in which additional analytical tests were performed, was used for the study of hypovitaminosis D and dysfunction and thyroid autoimmunity.

According to our data, waist circumference, waist-to-height ratio and body adiposity index are the adiposity measures that provided the best evaluation of the adiposity component for the metabolic syndrome in the Portuguese population. As the waist circumference is commonly used in the definition of the metabolic syndrome, and as the differences found by comparison with other adiposity measures were not relevant, its replacement was not justified. The cut-off points estimated for the Portuguese population (89 cm in women and 94 cm in men) were very similar to “European” cut-off points in women and “Europid” in men. The use of “European” cut-off points may

be more appropriate to avoid over-diagnosis in women. However, special attention should be given to waist circumference values greater than, or equal, to 94 cm in men.

Our study confirmed a high prevalence of the metabolic syndrome in Portugal. According to the definition of the Joint Interim Statement and according to "European" cut-off points, the prevalence of metabolic syndrome was 43.1% (45.7% in women and 39.8% in men. Differences in prevalence were found by districts and by area of residence. The districts of Vila Real and Leiria had a higher prevalence while the districts of Bragança and Beja had a lower prevalence. Participants living in non-urban areas had a higher prevalence of metabolic syndrome than those living in urban areas. The differences found are difficult to explain, and it is not possible to exclude differences in eating patterns, which were not evaluated in our study.

The metabolic syndrome prevalence was significantly higher in women and increased with age, as reported in previous national studies. Sedentary lifestyle and adiposity also had a positive association with the prevalence of the metabolic syndrome. In addition, socio-economic disadvantaged groups (retired, unemployed and domestic) also had a higher prevalence of the metabolic syndrome. The associations found, with a history of cardiovascular disease and diabetes type 2, as well as, with serum insulin levels and with HOMA score, reinforce the evidence on the increase of cardiovascular risk and the presence of insulin resistance in individuals with metabolic syndrome.

According to our results, the prevalence of hypovitaminosis D was very high in Portugal (37.7% and 47.9% of participants with deficiency and insufficiency, respectively) compared to populations in Europe and other parts of the world. Serum 25-hydroxy-vitamin D levels were significantly lower in the months of lesser

ultraviolet exposure (December-May period). Taking into account our findings, it is crucial to develop national policies to increase knowledge about the importance of vitamin D for health as well as to define strategies for the identification and treatment of vitamin D deficiency in at-risk groups. Serum 25-hydroxy-vitamin levels were positively associated with exercise and negatively with body mass index and serum triglyceride levels. Serum levels of 25-hydroxyvitamin D were also negatively associated with metabolic syndrome even after adjustment for age and sex. However, the association lost significance after further adjustment for body mass index. Our results reinforce the evidence suggesting that the effects of vitamin D on the metabolic syndrome are largely mediated by adipose tissue. In addition, negative associations with triglyceride components and blood pressure were observed, that remained after further adjustment for body mass index. The association observed between parathyroid hormone and body mass index, waist circumference and its component of metabolic syndrome support the evidence suggesting its contribution to obesity pathophysiology.

According to our results, the prevalence of thyroid dysfunction was 7.4% (4.9% and 2.5% for hypothyroidism and hyperthyroidism, respectively), with 72.7% of previously undiagnosed cases. In addition, dysfunction was much more prevalent in women (3.5 times higher) and subclinical forms predominated (63.9%). The high prevalence of hyperthyroidism in our sample may be related to a marginal iodine deficiency in the Portuguese population.

The prevalence of positivity for thyroid antibodies was 18.9% (11.9% and 15.1% for thyroid peroxidase and anti thyroglobulin antibodies respectively), with a higher expression in women (about 3 times higher).

No association of thyroid-stimulating hormone or thyroxine with the metabolic syndrome or its components was found. In addition, there were no associations of these hormones with the risk factors included in the metabolic syndrome or with the insulin resistance indicators. In contrast, triiodothyronine showed a positive association with the metabolic syndrome but not with its components. Additionaly, associations with serum triglyceride and insulin levels were also found.

Regarding thyroid antibodies, negative associations were found between thyroid peroxidase antibodies and both the metabolic syndrome and its triglyceride component. Meanwhile, thyroglobulin antibodies did not show any associations.

Resumo

A síndrome metabólica é uma entidade clínica que integra um *cluster* de factores de risco para a doença cardiovascular e diabetes tipo 2 e que partilham entre si mecanismos fisiopatológicos comuns entre os quais se destacam a resistência à insulina e a obesidade.

A inclusão de uma medida adiposa nas definições mais recentes da síndrome metabólica foi justificada pelo contributo da adiposidade para o risco de doença cardiovascular e de diabetes tipo 2 assim como para a fisiopatologia da síndrome. O tecido adiposo pode contribuir para a síndrome através de mecanismos relacionados com a resistência à insulina e a inflamação. As medidas de obesidade abdominal que melhor traduzem o tecido adiposo visceral poderão ser melhores indicadores do risco associado a adiposidade. Entre estas medidas da obesidade abdominal o perímetro da cintura tem-se destacado, sendo usada nas mais recentes definições da síndrome metabólica. Vários pontos de corte têm vindo a ser propostos para a definição da componente perímetro da cintura da síndrome metabólica. De acordo com a mais recente definição da síndrome metabólica foram propostas duas alternativas de pontos de corte para as populações europeias ("European" e "Europid"). Estes dois conjuntos de pontos de corte do perímetro da cintura, estimados na população caucasóide, seriam aqueles que melhor identificavam valores do índice de massa corporal maiores ou iguais a 25 Kg/m^2 (80 cm na mulher and 94 cm no homem) e 30 kg/m^2 (88 cm na mulher and 102 cm no homem). Estes dois diferentes pontos de corte também foram adoptados pela Organização Mundial de Saúde para a estratificação do risco

metabólico associado à obesidade abdominal (risco elevado e risco muito elevado respectivamente para pontos de corte mais baixos e mais altos). Porém, para além das mais recentes recomendações sobre a síndrome metabólica terem sugerido o uso de pontos de corte do perímetro da cintura personalizados para populações específicas, um número crescente de estudos, com origem em diferentes países e étnias, tem vindo a estimar pontos de corte específicos para a identificação do risco associado à síndrome metabólica. Em Portugal a prevalência da obesidade abdominal, definida de acordo com os pontos de corte propostos pela Organização Mundial de Saúde, é bastante elevada, nomeadamente na mulher. Tendo em conta estes dados sobre a prevalência nacional da obesidade abdominal, poderá ser interrogada a adequação para a população portuguesa dos pontos de corte do perímetro da cintura propostos pela mais recente definição da síndrome metabólica. Porém, ainda não foi realizado em Portugal qualquer estudo sobre o assunto. Para além disso, vários estudos têm vindo a sugerir que outras medidas da obesidade abdominal, nomeadamente a razão cintura-altura, poderão ser mais adequadas do que o perímetro da cintura para a estimativa do risco associado à síndrome metabólica.

A síndrome metabólica tem uma elevada prevalência em Portugal, quando comparada com a maior parte dos países Europeus, Estados Unidos da América e outras regiões do globo. De acordo com vários estudos transversais, a prevalência nacional da síndrome metabólica variou entre 24% e 28.4%, 42% e 66% e 37.2% e 69.4%, de acordo com as definições *Adult Treatment Panel III*, *International Diabetes Federation e Joint Interim Statement*, respectivamente. A elevada prevalência nacional da obesidade, da diabetes tipo 2 e da hipertensão arterial podem ter contribuído para

estes números, assim como os comportamentos relacionados com o estilo de vida e características socio-económicas.

Vários estudos, em todo o mundo, têm encontrado diferenças da prevalência da síndrome metabólica entre populações rurais e urbanas. Nenhum dos estudos realizados até agora em Portugal abordou as diferenças entre as populações não urbanas e urbanas.

O sistema endócrino tem um importante papel na regulação do equilibrio energético, da pressão arterial e da sensibilidade à insulina assim como no metabolismo da glicose e dos lípidos. Diversas doenças endócrinas têm sido associadas com a síndrome metabólica e o risco de doença cardiovascular e de diabetes tipo 2. Entre elas destacam-se pela sua actualidade e prevalência a hipovitaminose D e a disfunção e autoimunidade tiroideias. Em Portugal, para além dos estudos sobre a prevalência da hipovitaminose D realizados em grupos específicos, a hipovitaminose D e a disfunção e a autoimunidade tiroideias têm sido pouco estudadas, nomeadamente no que se refere ao risco cardiovascular e de diabetes tipo 2.

O principal objectivo desta tese, foi estudar a síndrome metabólica e as suas determinantes em Portugal com um foco especial na influência da obesidade e de patologias endócrinas frequentes, incluindo a hipovitaminose D e a disfunção e autoimunidade tiroideias.

Para atingir este objectivo, foram estabelecidas várias tarefas específicas. Primeiro, foram identificadas as medidas adiposas que melhor estimavam o risco associado à síndrome metabólica numa amostra da população portuguesa adulta, assim como os respectivos pontos de corte (Paper I). De seguida, foi analisada a prevalência da

síndrome metabólica e das suas determinantes, na mesma população tendo em conta os pontos de corte identificados no passo anterior e a distribuição da população por distritos, NUTS II e residência urbana ou não urbana (Paper II). Posteriormente, foi analisada numa subamostra da mesma população, a prevalência da hipovitaminose D, assim como as determinantes dos níveis séricos da 25-hidroxi-vitamina D e da hormona paratiroideia e as suas possíveis associações com a síndrome metabólica e as suas componentes (Paper III). Por fim, na mesma subamostra, foi analisada a prevalência da disfunção tiroideia e da positividade dos anticorpos tiroideus e foram estudadas as associações da *thyroid-stimulating hormone*, das hormonas tiroideias e dos anticorpos tiroideus com a síndrome metabólica e as suas componentes (Paper IV).

Para a concretização destes objectivos foi usada uma amostra de adultos incritos nos cuidados de saúde primários (estudo PORMETS). Em cada um dos 18 distritos de Portugal continental foram incluídos 2 centros de saúde, um da capital e outro representativo de área não urbana. Em cada centro de saúde 120 participantes foram selecionados aleatoriamente a partir da listagem dos utentes inscritos. Foram incluídos 4095 participantes entre Fevereiro de 2007 e Julho de 2009. Uma subamostra incluindo 500 participantes selecionada aleatoriamente a partir da amostra inicial do estudo PORMETS, em que foram feitos exames analíticos adicionias, foi usada para o estudo da hipovitaminose D e da disfunção e autoimunidade tiroideias.

De acordo com os nossos resultados, o perímetro da cintura, a razão cintura-altura e o *body adiposity índex* são as medidas adiposas que melhor estimam a componente adiposa da síndrome metabólica. Como o perímetro da cintura é habitualmente usado na definição da síndrome metabólica e porque as diferenças encontradas por

comparação com as outras medidas da adiposidade foram pouco relevantes, não se justificou a sua substituição. Os pontos de corte do perímetro da cintura estimados para a população portuguesa (89 cm na mulher e 94 cm no homem) eram muito semelhantes aos pontos de corte "European" na mulher e "Europid" no homem. O uso dos pontos de corte "European" poderá ser mais apropriado para evitar o sobrediagnóstico na mulher. Porém, uma especial atenção deverá ser dada a valores do perímetro da cintura maiores ou iguais a 94 cm no homem.

O nosso estudo confirmou uma elevada prevalência da síndrome metabólica em Portugal.

De acordo com a definição da *Joint Interim Statement* e de acordo com pontos de corte "European" a prevalência da síndrome metabólica foi de 43.1% (45.7% nas mulheres e 39.8% nos homens). Foram encontradas diferenças na prevalência por distritos e por área de residência. Os distritos de Vila Real e Leiria apresentaram maior prevalência e os distritos de Bragança e Beja menor prevalência. Os participantes residentes em áreas rurais apresentaram maior prevalência do que os residentes em áreas urbanas. As diferenças encontradas são de difícil explicação, não se podendo excluir diferenças nos hábitos alimentares, que não foram avaliados no nosso estudo.

A prevalência foi significativamente maior no sexo feminino e aumentou com idade, como referido em estudos nacionais anteriores. O sedentarismo e a adiposidade também apresentaram uma associação positiva com a prevalência da síndrome metabólica. Para além disso, os grupos mais desfavorecidos do ponto de vista sócio-económico (reformados, desempregados e domésticas), também apresentaram maior prevalência da síndrome metabólica. As associações encontradas com os antecedentes

de doença cardiovasculares e de diabetes tpo 2 assim como com os níveis séricos de insulina e com o HOMA, reforçam a evidência sobre o aumento do risco cardiovascular e a presença de insulinorresistência nos indivíduos com síndrome metabólica.

De acordo com os nossos resultados a prevalência de hipovitaminose D era muito elevada em Portugal (37.7% e 47.9% dos participantes com deficiência e insuficiência, respectivamente) por comparação com populações europeias e de outras partes do mundo.

Os níveis de 25-hidroxi-vitamina D eram ainda significativamente mais baixos nos meses com menor exposição ultra-violeta (período Dezembro-Maio). Tendo em conta os nossos resultados torna-se crucial o desenvolvimento de políticas nacionais para aumentar o conhecimento sobre a importância da vitamina D para a saúde assim como definir estratégias para a identificação e tratamento da deficiência da vitamina D em grupos de risco.

Os níveis séricos da 25-hidroxi-vitamina D apresentaram uma associação positiva com o exercício físico e negativa com o índice de massa corporal e os níveis séricos de triglicéridos. Os níveis séricos de 25-hidroxivitamina D apresentaram ainda uma associação negativa com a síndrome metabólica após ajustamento para idade e sexo. Porém a associação perdeu significância após ajustamento adicional para o índice de massa corporal. Estes resultados vêm reforçar a evidência que sugere que os efeitos da vitamina D na síndrome metabólica são mediados em grande parte pelo tecido adiposo. Para além disso foram observadas associações negativas com os componentes triglicéridos e pressão arterial da síndrome metabólica que se mantiveram após

ajustamento adicional para o índice de massa corporal. A associação da hormona paratiroideia com o índice de massa corporal e com o perímetro da cintura e respectivo componente da síndrome metabólica vem apoiar a evidência que sugere o seu contributo para a obesidade.

De acordo com os nossos resultados a prevalência da disfunção tiroideia foi de 7.4% (4.9% e 2.5% para o hipotiroidismo e hipertiroidismo, respectivamente), com 72.7% dos casos não diagnosticados previamente. Para além disso, a disfunção foi muito mais prevalente nas mulheres (3.5 vezes superior) e as formas subclínicas predominaram (63.9%). A elevada prevalência do hipertiroidismo na nossa amostra pode estar relacionada com uma deficiência marginal de iodo na população portuguesa. A prevalência da positividade para os anticorpos tiroideus foi de 18.9% (11.9% e 15.1% para os anticorpos anti-peroxidase tiroideia e anti-tireoglobulina respectivamente), com uma maior expressão nas mulheres (cerca de 3 vezes superior).

Não foi encontrada qualquer associação da *thyroid-stimulating hormone* ou da tiroxina com a síndome metabólica ou com as suas componentes. Para além disso, não se encontraram associações destas hormonas com os factores de risco incluídos na síndrome metabólica ou com os indicadores de insulino-resistência. Em contrapartida, a triiodotironina apresentou uma associação positiva com a síndrome metabólica mas não com as suas componentes. Para além disso, foram ainda encontradas associações com os níveis séricos dos triglicéridos e da insulina.

No que respeita aos anticorpos tiroideus encontraram-se associações negativas com a síndrome metabólica e com sua acomponente triglicéridos para os anticorpos anti-

peroxidase tiroideia. Os anticorpos anti-tireoglobulina não apresentaram quaisquer associações.

I. Background

1. Metabolic syndrome: concept, definitions and controversies

The concept of metabolic syndrome (MetS) results from the co-occurrence of cardiovascular disease (CVD) and type 2 diabetes risk factors.

The MetS, from an epidemiological and clinical point of view, is since 2009 an entity with a clear and consensual definition and integrates five independent components [1].

Several scientific organizations, including the World Health Organization (WHO) [2], the European Group for Study of Insulin Resistance (EGIR) [3], the National Heart, Lung, and Blood Institute (NHLBI) [1, 4-8], the American Heart Association (AHA) [1, 4-8], the American Diabetes Association (ADA) [4, 5, 8], the American Association of Clinical Endocrinologists (AACE) [9], the American College of Endocrinology (ACE) [9], the International Diabetes Federation (IDF) [1, 10, 11], the World Heart Federation (WHF) [1], the International Atherosclerosis Society (IAS) [1], and the International Association for the Study of Obesity (IASO) [1], proposed widely used epidemiological definitions of the MetS, (Tables 1, 2 and 3).

Table 1. Metabolic Syndrome definitions

	WHO (1998) [2]	EGIR (1999) [3]	ATP III (2001) [4]
Insulin resistance	IR: glucose uptake < lower quartile clamp)	IR: glucose uptake < lower quartile (clamp) or fasting hyperinsulinaemia	Not included
Glucose	Impaired glucose regulation * or diabetes	Fasting plasma glucose ≥ 6.1 mmol/L (110 mg/dL) and non diabetic	Fasting plasma glucose ≥ 6.1 mmol/L (110 mg/dL) or diabetes
Triglycerides	≥ 1.7 mmol/L (150 mg/dL)	> 2.0 mmol/L (180 mg/dL) or treatment for dyslipidaemia	≥ 1.7 mmol/L (150 mg/dL)
HDL cholesterol	M < 0.9 mmol/L (35 mg/dl) W < 1.0 mmol/L (39 mg/dl)	< 1.0 mmol/L (40 mg/dL) or treatment for dyslipidaemia	M < 1.04 mmol/L (40 mg/dL) W < 1.30 mmol/L (50 mg/dL)
Blood pressure	≥ 160/90 mmHg	≥ 140/90 mmHg or treatment for hypertension	≥ 130/85 mmHg
Abdominal obesity	M: WHR > 0.90 W: WHR > 0.85 and /or BMI > 30 Kg/m^2 **	M: WC ≥ 94 cm W: WC ≥ 80 cm	M: WC > 102 cm W: WC > 88 cm
Microalbuminuria	UAE rate ≥ 20 µg/min or UACR ≥ 20 mg/g	Not included	Not included
MetS criteria	IR and/or glucose component and ≥ 2 of 4 possible components: a. Atherogenic dyslipidemia ***; b. Blood pressure; c. Abdominal obesity; d. Microalbuminuria.	IR and ≥ 2 of 4 possible components: a. Glucose; b. Dyslipidaemia ***; c. Blood pressure; d. Abdominal obesity.	≥ 3 of 5 possible components: a. Glucose b. Triglycerides c. HDL cholesterol; d. Blood pressure; e. Abdominal obesity.

ATP III, National Cholesterol Education Program (NCEP) Adult Treatment Panel III; M, men; W, Women; IR, insulin resistance; HDL, high density cholesterol; WC, waist circumference; WHR, waist-to-hip ratio; BMI, body mass index; UAE, urinary albumin excretion; UACR, urinary albumin-to-creatinine ratio; MetS, metabolic syndrome.

* Impaired glucose regulation includes impaired glucose tolerance (IGT) and impaired fasting glycaemia (IFG). ** 10-15% lower for non-Caucasian. *** Triglycerides and/or HDL cholesterol.

Table 2. Metabolic Syndrome definitions

	AACE (2003) [9]	AHA/NHLBI/ADA (2004) [8]	AHA/NHLBI (2005) [6]
Glucose	Fasting plasma glucose ≥ 110 mg/dL and < 126 mg/dL (excluding diabetes) or IGT *	Fasting plasma glucose ≥ 100 mg/dL (including diabetes)	Fasting plasma glucose ≥ 100 mg/dL (including diabetes) or drug treatment
Triglycerides	> 1.7 mmol/L (150 mg/dL)	≥ 1.7 mmol/L (150 mg/dL)	≥ 1.7 mmol/L (150 mg/dL) or drug treatment
HDL cholesterol	M < 40 mg/dL W < 50 mg/dL	M < 1.04 mmol/L (40 mg/dL) W < 1.30 mmol/L (50 mg/dL)	M < 1.03 mmol/L (40 mg/dL) W < 1.30 mmol/L (50 mg/dL) or drug treatment
Blood pressure	> 130/85 mmHg	≥ 130/85 mmHg	≥ 130/85 mmHg or drug treatment
Abdominal obesity	M: WC > 102 cm W: WC > 88 cm or BMI > 25 Kg/m^2	M: WC > 102 cm W: WC > 88 cm	M: WC ≥102 cm W: WC ≥ 88 cm
MetS criteria	Presence of a Risk factor for IRS ** and ≥ 2 of 4 possible components: a. Glucose; b. Triglycerides; c. HDL cholesterol; d. Blood pressure.	≥ 3 of 5 possible components: a. Glucose; b. Triglycerides; c. HDL cholesterol; d. Blood pressure; e. Abdominal obesity.	≥ 3 of 5 possible components: a. Glucose; b. Triglycerides; c. HDL cholesterol; d. Blood pressure; e. Abdominal obesity.

IRS, Insulin resistance syndrome; M, men; W, women; HDL, high-density cholesterol; WC, waist circumference; BMI, body mass index; MetS, metabolic syndrome.

* IGT: Plasma glucose 120 minutes post-glucose challenge (75 g) ≥ 140 mg/dl and ≤ 200 mg/dl.

** Risk factors for IRS: Abdominal obesity; sedentary lifestyle; age >40 years; non-Caucasian ethnicity; family history of type 2 diabetes, hypertension or cardiovascular disease; history of glucose intolerance or gestational diabetes; acanthosis nigricans; polycystic ovary syndrome; non-alcoholic fatty liver disease.

Table 3. Metabolic Syndrome definitions

	IDF (2005) [10]	JIS (2009) [1]
Glucose	Fasting plasma glucose ≥ 100 mg/dL (5.6 mmol/L) or type 2 diabetes	Fasting plasma glucose ≥ 100 mg/dL (5.6 mmol/L) or drug treatment
Triglycerides	≥ 150 mg/dL (1.7 mmol/L) or drug treatment	≥ 150 mg/dl (1.7 mmol/L) or drug treatment
HDL cholesterol	M < 40 mg/dL (1.03 mmol/L) W < 50 mg/dl (1.29 mmol/L) or drug treatment	M < 40 mg/dL (1.0 mmol/L) W < 50 mg/dl (1.3 mmol/L) or drug treatment
Blood pressure	≥ 130/85 mmHg or drug treatment	≥ 130/85 mmHg or drug treatment
Abdominal obesity	Ethnicity-specific* Europids criteria: M: WC ≥ 80 cm W: WC ≥ 94 cm or BMI ≥ 30 Kg/m^2	Population- and country-specific definitions **
MetS criteria	Abdominal obesity and ≥ 2 of 4 possible components: a. Glucose; b. Triglycerides; c. HDL cholesterol; d. Blood pressure.	≥ 3 of 5 possible components: a. Glucose b. Triglycerides c. HDL cholesterol; d. Blood pressure; e. Abdominal obesity.

JIS, Joint Interim Statement; M, men; W, women; HDL, high density cholesterol; WC, waist circumference; BMI, body mass index; MetS, metabolic syndrome.

*Other Ethnic groups: South Asians, Chinese and Central Americans (Men ≥ 90 cm / Women ≥ 80 cm); Japanese (Men ≥ 85 cm / Women ≥ 90 cm); Saharan Africans, Eastern Mediterranean and Middle East (Arab) populations - use Europids criteria until more specific data are available.

** Population- and country- specific groups: Europid/IDF (Men ≥ 94 cm / Women ≥ 80 cm); European/European Cardiovascular Societies (Men ≥ 102 cm / Women ≥ 88 cm); Caucasian /WHO (Men ≥ 94 / 102 cm/ Women ≥ 80 / 88 cm); United States/ATP III (Men ≥ 102 cm / Women ≥ 88 cm); Asian (including Japanese)/IDF (Men ≥ 90 cm / Women ≥ 80 cm); Asian/WHO (Men ≥ 90 cm / Women ≥ 80 cm); Japanese/Japanese Obesity Society (Men ≥ 85 cm / Women ≥ 90 cm); China/Cooperative Task Force (Men ≥ 85 cm / Women ≥ 80 cm); Middle East, Mediterranean/IDF (Men ≥ 102 cm / Women

≥ 88 cm); Sub-Saharan African/IDF (Men ≥ 94 cm / Women ≥ 80 cm); Ethnic Central and South American/IDF (Men ≥ 90 cm / Women ≥ 80 cm).

The WHO definition of MetS [2] (Table 1) was the first with global impact. A set of criteria was proposed for a clinical diagnosis of the MetS, including impaired fasting glycaemia.

(IFG), impaired glucose tolerance (IGT) or diabetes mellitus and/or insulin resistance (IR) and at least 2 of 4 other possible components: blood pressure (BP), dyslipidaemia [triglycerides and/or high-density lipoprotein (HDL) cholesterol], abdominal obesity (AO) and microalbuminuria. Although this group valued the central role of IR in the pathophysiology of the MetS, it was considered more appropriate to use the term "metabolic syndrome" instead of "syndrome X", as it was previously used [12], because, in the later definition, AO was not included.

The EGIR [3] consensus criticized the term MetS proposed by the WHO and proposed to retain the term insulin resistance syndrome (IRS) arguing that IR was the central element. The IRS was thus defined by the presence of IR or fasting hyperinsulinaemia (the highest 25% fasting insulin concentrations of a nondiabetic population) and two or more other components, including hyperglycaemia, BP, dyslipidaemia (triglycerides or HDL cholesterol) and AO.

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) [4, 5] recommendations (Table 1) proposed a clinical definition of the MetS including 5 components: waist circumference (WC), triglycerides, HDL cholesterol, BP and glucose; the diagnosis of the MetS was made when 3 or more of the risk components

were present. The ATP III panel, individualized the two components of the atherogenic dyslipidaemia and recognized the phenomenon of clustering of metabolic risk factors and the link to IR, but it was not conclusive about the pathogenic mechanisms of the syndrome, because the mechanisms underlying the link of IR with the CVD risk factors were not fully understood; as such the IR component was excluded from the definition.

The AACE and the ACE statement [9] (Table 2), adopted the lipidic and BP criteria of ATP III but proposed changes to other components of the definition. According to them, individuals with diabetes should be excluded and changes were proposed to the glucose component. Postprandial glucose was included as an alternative to fasting glucose, due to its limitations [13, 14]. Unlike the definitions of WHO, EGIR and ATP III, in the AACE definition, the presence of at least one of several IRS risk factors was mandatory for diagnosis. The following IRS risk factors were considered: AO; sedentary lifestyle; age >40 years; non-Caucasian ethnicity; family history of type 2 diabetes, hypertension or CVD; history of glucose intolerance or gestational diabetes; acanthosis nigricans; polycystic ovary syndrome; non-alcoholic fatty liver disease. Like EGIR, the AACE statement preferred to use the term IRS instead of MetS, adopted by WHO and ATP III, to reinforce the central pathophysiological and unifying role of IR and compensatory hyperinsulinemia.

In 2004 [8], a report of the AHA, NHLBI and ADA Conference on Scientific Issues Related to Management of MetS (Table 2) was published. According to the follow-up report on the diagnosis of diabetes mellitus of the ADA [15] published in 2003, it was proposed a new cut-off point for fasting plasma glucose of 100 mg/dl, applicable to define the glucose component of the MetS according to ATP III definition.

In 2005, the AHA and NHLBI reaffirmed the utility of ATP III criteria, with minor modifications [6, 7] (Table 2). The new cut-off point for fasting plasma glucose (100 mg/dL) was adopted. This statement, according to previous positions, maintained the term MetS rather than IRS. According to these recommendations, the AO [16] and IR [12, 17-19] were considered as predominant underlying risk factors for MetS and AO was stated as the main modifiable risk factor. Compared with the AHA, NHLBI and ADA, 2004 definition, the subjects on drug treatment for any of the components of the syndrome were included for diagnostic purposes.

According to the 2005 IDF definition [10, 11] (Table 3), AO had a key role in the pathogenesis of MetS and, for the first time, its presence was considered mandatory for the diagnosis of MetS. In addition, and in a much more extensive and clear way, the ethnic differences regarding the cut-off points for the definition of the AO component were valued. MetS was defined by the AO component plus any two of four additional traits: dysglycaemia, raised BP, elevated triglyceride levels and low HDL cholesterol levels.

Finally, in 2009, a Joint Interim Statement (JIS) definition [1] (Table 3) used the same components and the same methodology of the AHA and NHLBI statement of 2005 [6, 7], for diagnosis of MetS. Following the recommendations of the IDF to individualize WC cut-off points due to ethnic and geographic specificities, different cut-off points were proposed according to populations and ethnicity. With regard to the European Caucasian population, two different cut-off points were proposed: "Europid" according to IDF [10] and “European "according to the European Cardiovascular Societies [20].

2. The adiposity component: pathophysiological and cut-off points controversies

The contribution of obesity to CVD [21-27] and diabetes risk [28-33] justified, as previously stated, the inclusion of an adiposity measure, in the most frequently used definitions of MetS. However, the relevance of the adipose component for the syndrome and its definition has been the subject of some controversy [2-4, 6, 9, 11]. In fact, although obesity and IR play a key role in the pathophysiology of the MetS, their relative importance has been the subject of much debate [3, 9].

Total body and abdominal fat are distributed principally by two compartments, the subcutaneous adipose tissue and the visceral adipose tissue (VAT) [34, 35], and there is evidence that VAT may have a stronger impact than total body fat on IR [36-43]. Total body fat and VAT may be estimated by several proxy anthropometric indicators, including body mass index (BMI) on the one hand, and WC, waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR) [44], on the other hand. Despite its good correlation with total body fat, BMI is only moderately correlated with VAT [45]. In addition, AO anthropometric measures present stronger associations with VAT than total body fat measures [46]. Furthermore, AO anthropometric measures (WC, WHR and WHtR) have also shown to be better predictors of CVD risk and mortality [25, 47-55] and diabetes risk [28-31], than BMI. Two systematic reviews and meta-analysis [56, 57] even suggested that WHtR was modestly superior to WC for CVD risk assessment. In addition, WHtR may be a better predictor of diabetes than other AO measures [58]. Nevertheless, the differences between these several measures may not be clinically relevant [54]. Another measure of total body adiposity, the body adiposity index (BAI), that is calculated from

hip circumference (HC) and height, has been proposed [59]. BMI and BAI performed similarly when estimating the percentage of fat [46]. However, BAI showed a lower correlation with VAT and the homeostatic model assessment (HOMA) [60, 61], and may not be as good as BMI in predicting CVD risk [46, 62-66], all-cause and CVD mortality [62], and diabetes risk [63].

According to the WHO [2] proposal, AO (or central obesity, as it was called in the publication) was included as a component of the MetS, and was defined by WHR (> 0.90 in males and >0.85 in females) and/or BMI > 30 Kg/ m^2. Although the association of AO with IR [67, 68] and other components of the syndrome [12, 17, 69-77] had already been described, it was with this WHO publication that AO gained prominence as a fundamental part of the syndrome. In fact, AO was not included in the original "Syndrome X" described in 1988 by Reaven [12, 17, 18]; however, it was posteriorly included in the "deadly quartet" of Kaplan [69] and in the "syndrome X plus" of Zimmet [77], in 1989 and 1991, respectively.

The EGIR definition [3], adopted and confirmed the WC as a measure of the adipose component of the MetS. They preferred to use the WC as an anthropometric measure of the abdominal adipose tissue because it was simpler to measure than WHR, and WC better correlated with intra-abdominal VAT accumulation than BMI or WHR [78, 79]. The WC cut-off points (94 cm for men and 80 cm for women) were proposed based in a cross-sectional study (MORGEN project) [80, 81] conducted by Lean and Han in 1993-1995, including 5887 men and 7018 women aged 20–59 years from the general population of Netherlands. These cut-off points, previously identified in 1992 by the

same investigators in a population sample of Glasgow [82], were the ones that better identified subjects with a BMI ≥ 25 Kg/m^2.

The NCEP ATP III [4, 5] definition also included WC cut-off points. These cut-off points (88 cm and 102 cm, respectively in women and men) identified subjects with a BMI≥ 30 Kg/m^2 [83, 84]. The choice of EGIR and ATP III cut-off points was based on the same publications of Lean and Han [80-82].

In 2003, for the first time in a clear way, the AACE recommendations [9] highlighted the need for adjustment of anthropometric measures of adiposity to ethnicity. Cut-off points 10 to 15% lower were suggested for non-Caucasians. In addition, the BMI was recovered and was added to the WC as an anthropometric measure of adiposity [85]. According to the AACE definition, BMI and WC could be used alternatively as anthropometric measurements of adiposity. The cut-off points of 25 Kg/m^2 for BMI and of 102 cm and 88 cm for WC, respectively in men and women, were proposed.

In 2004, the NHLBI and the AHA published a report on the MetS [86]. According to this report, the pathogenesis of the MetS included three potential etiological categories: obesity and disorders of adipose tissue; IR; and a constellation of independent factors that mediate specific components of the MetS. Other factors, like ageing, proinflammatory state, and hormonal changes, were implicated as contributors as well, but the independent role of AO was stressed in the report.

In 2005, the AHA and NHLBI [6, 7] reinforced ATP III recommendations by maintaining the same WC cut-off points. However, lower WC cut-off points were suggested for populations from South Asia, China, Japan, and other Asian countries [87-91].

Also in the same year, the IDF [10, 11] proposed new diagnostic criteria for MetS. According to IDF, AO, estimated by WC, played a key role in the early stages of MetS pathogenesis. This assumption was supported by evidence of a strong association of WC with IR and the various components of MetS and with the risk of CVD. The presence of AO was considered as a precondition for the identification of MetS and the proposed cut-off points could vary between countries/ethnic groups ("Europid", South Asians, Chinese, Japanese, Ethnic-South and Central Americans and Sub-Saharan Africans). In the USA population, the ATP III cut-off points were maintained. In the "Europid" group (white people of European origin) cut-off point of 94 cm in males and 80 cm in females were proposed; these cut-off points were based on cross-sectional data from the Netherlands studies [80-82] and were the best values for identifying people with increased adiposity, defined as a BMI of ≥25 kg/m^2 or WHR ≥0.90 for men and ≥ 0.85 for women. The IDF also suggested the need to conduct longitudinal studies with risk assessment of CVD and diabetes in various ethnic groups to estimate the most appropriate WC cut-off points.

Finally, in 2009, the JIS [1] proposed a harmonizing definition of the MetS, suggesting the use of national or regional WC cut-off points to define the adiposity component of MetS. Two sets of cut-off points were proposed for European individuals: "Europid" (94 cm in men and 80 cm in women) and "European" (102 cm in men and 88 cm in women), according to the IDF [10, 11] and the European Cardiovascular Societies [20], respectively.

As stated before, these two sets of cut-off points, estimated in Caucasian populations, were those that best identified BMI values greater than or equal to 25 kg/m^2 (80 cm in

women and 94 cm in men) and 30 kg/m2 (88 cm women and 102 cm in men) [80-82]. Its use was reinforced by a WHO technical report on obesity that confirmed these sex-specific WC cut-off points to identify the risk of metabolic complications associated with obesity in Caucasians [92]. The risk was classified as increased (or high) or substantially increased (or very high), according to the lower and higher WC cut-off points respectively [93].

An increasing number of studies, from several countries and ethnicities, reported different values for the cut-off points of WC and other anthropometric measures of AO that best estimated CVD and diabetes risk [94, 95].

In Portugal, where the prevalence and trend of obesity has risen to "pandemic" proportions [96-102], following what is happening in Europe [103-105] and worldwide [106, 107], several studies were carried out to evaluate the prevalence [97, 99] and incidence [98] of AO.

According to the EPIPorto study [108], a cohort study conducted in the city of Porto in the period of 1999-2003, the prevalence of AO (> 88 cm in women and > 102 cm in men) was 40.1% and 16.6 %, in women and men, respectively.

In a national study conducted between January 2003 and January 2005 [97], 49.8% of the participants (47.8% and 52.1%, respectively in women and men) presented WC ≥ 80 cm and WC ≥94 cm, respectively for women and men. For higher cut-off points (WC ≥ 88 cm and WC ≥102 cm, respectively for women and men) a prevalence of 24.9 % was found (24.6% and 25.3%, respectively for women and men). According to another Portuguese study [99], in which the cut-off points for AO were defined as WC values greater than 88 and 102 cm, respectively in women and men, women had a higher prevalence of AO than men (37.9% and 19.3%, respectively) in the participants

aged 18-64 years. Older adults (≥ 65 years) presented higher values (69.7% and 32.1%, respectively in women and men).

Compared with other European countries [93, 109-124] (Table 4), and although the results in Portugal are quite diverse [97, 99], higher prevalence estimates for AO were observed in women, following the trend of most studies [93, 110-114, 116-119, 123]. The national prevalence of AO, according to available estimates, is high compared to several European countries. Taking into account the worst estimates, the prevalence of AO in Portugal may be similar to that of the USA [125]. In addition, compared with mean worldwide estimates, national results were also high [126].

Table 4. Prevalence of abdominal obesity in Europe and Worldwide

Country	Study date	Age (years)	Men (94 cm)	Men (102 cm)	Women (80 cm)	Women (88 cm)
Portugal [108]	1999-2003	≥ 18		16.6		40.1
Portugal [97]	2003-2005	18-64	52.1	25.3	47.8	24.6
Portugal [99]	2008-2009	≥ 18		19.3		37.9
Spain [111]	2008-2010	≥ 18		31.7		39.2
France [112]	2006	≥ 16		49.5		58.2
France [113]	2006	≥18	42.3		51.6	
France [114]	2013	30-69	41.6		48.5	
Italy [115]	2007	≥ 18		57.0		56.4
Greece [116]	2003	20-70	27.7	26.6	20.7	35.8
England [93]	2008	18-67	62.8	35.7	66.8	43.9
Ireland [117]	2011	18-64	22.5	31.1	26.6	37.3
Germany [118]	2008-2011	18-79	54.2	31.3	56.9	36.4
Belgian [119]	2014	40-70	37.2	28.7	50.0	23.6
Denmark [120]	2011-2012	Adults	40.2		35.7	
Norway [121]	2006-2008	≥ 20	31.9	31,9	23.7	55.9
Sweden [122]	2006	18-65	29.9	32.7	26.7	42.5
Poland [123]	2013-2014	≥ 20	32.2	27.2	45.7	21.7
Romania [110]	2012-2014	20-79	68.9		73.9	
Russia [109]	2000	18-90		6.3		29.0
Europe [124]	1992-2000	25-70		23.1		24.1
USA [125]	2007-2008	≥ 20		43.4		61.6
Worldwide [126]			56.0	29.0	71.0	48.0

Taking into account data on AO in the Portuguese population, and the differences found in relation to other populations, it can be questioned if any of the two levels of WC cut-off points usually proposed are appropriate to the Portuguese reality. In addition, although the most recent MetS definitions have adopted WC as the anthropometric measure of adiposity, several other studies have suggested the use of other adipose measures, namely WHtR [56-58].

There are few longitudinal studies that analyse the relationship between the different anthropometric measures of AO and the risk of CVD and diabetes by ethnic groups and by sex [1]. In Portugal, no longitudinal study has yet been conducted with the objective of determining WC cut-off points as a function of the risk of CVD and diabetes.

In order to choose the best anthropometric measure to estimate adipose tissue and respective cut-off points, several methodological procedures may be used in cross-sectional studies. One of the proposed methods uses receiver operating characteristic (ROC) curves to compare the ability of anthropometric measures of AO to identify the MetS and to estimate the cut-off points that better identify MetS and corresponding CVD and diabetes risk, using the point that maximizes the sensitivity plus specificity [94, 95]. However, the strength of association of measures of AO with each of the components of MetS may vary according to the population studied [1]. In addition, the predictive value of AO in the evaluation of the risk of CVD [127] and diabetes [128] varies according to its severity.

Although several cross-sectional studies have addressed AO, no national study has yet been conducted in order to estimate and to compare the cut-off points for several AO anthropometric measures that better identify the MetS.

3. Metabolic syndrome: prevalence in the World, Europe and Portugal

MetS is a clinical entity that has reached a large and growing world dimension [129]. This is not independent of the increasing prevalence of obesity, diabetes and sedentary lifestyles, on a global scale.

In Portugal, the CVD risk factors included in the MetS definition by the JIS statement [1] are highly prevalent. In fact, the national prevalence of overweight and obesity in Portugal is high [96-99] and shows a growing trend [100-102]. In the adult national population, only less than 50% of the individuals present normal weight [102]. The prevalence of hypertension and type 2 diabetes is also high in Portugal (42.2% and 11.7%, respectively) [130, 131].

As expected, the prevalence of MetS is high in the Portuguese population [132-135]. According to the various cross-sectional studies available, the prevalence of MetS ranged from 24.0% to 28.4%, 42% to 66%, and 37.2% to 69.4%, according to the ATP III, IDF criteria and JIS (WC European criteria), respectively.

Comparing with other European countries, the national prevalence of the MetS is relatively higher than in Southern [136-149] (Table 5), Northern [150-159] (Table 6), Western [160-172] (Table 7), and Eastern Europe (Table 8) [89, 173-190], with the exception of Greece [143], Spain [137], Germany [168], Luxembourg [166], Croatia [175], Romania [178], Poland [186] and Turkey [190]. According to two reports [191, 192] resulting from collaboration between several European centers involved in cross-sectional studies, the prevalence of MetS in Europe according to the ATP III definition was 23.9% to 25.9% in men and 23.4% to 24.6% in women.

Compared with other regions of the world, including North [193-198], Central [199] and South [200-204] America, Asia and Pacific [205-224], Middle East [225-241] and Africa [242-251], Portugal presents, in general, a higher prevalence of the MetS. In addition, Portugal presents estimates similar or slightly higher than those found in the United States [193-195, 197, 198] and in Brazil [202].

Several studies conducted around the world have shown differences between urban and rural populations (Table 9). In some cases, there was a greater prevalence in urban areas [208, 223, 252-256] while in others the opposite occurred [215, 257]. The differences found between urban and rural populations have been partially explained by demographic and ethnic factors, as well as differences in eating habits and physical activity, among others [208, 215, 223, 241, 252-264], but studies carried out so far in Portugal, did not address the differences between urban and non-urban populations.

Table 5. Metabolic syndrome crude prevalence – Southern Europe

Country	Study date	Sample size	Age range	MetS definition	Men (%)	Women (%)	Total (%)
Portugal [132]	2005-2008	1433	≥ 18	ATP III	18.7	27.2	24.0
				IDF	37.8	44.4	41.9
				JIS (102/88 cm)	32.4	40.1	37.2
Portugal [133, 135]	2006-2007	16856	18-96	ATP III	27.3	29.5	28.4
				IDF	60.1	70.4	65.5
				JIS (102/88 cm) [b]	65.6	72.8	69.4
Portugal [134]	2008-2009	5167	20-79	IDF			41.5
Spain [136]	2004-2008	2459	20-92	IDF	19.2	12.1	15.0
				JIS (102/88 cm)	14.9	11.1	12.7
Spain [137]	2009-2010	4727	18-90	IDF	40.2	37.4	
				JIS (102/88 cm)	34.0	33.9	
Spain [138]	2008-2010	11149	≥18	JIS (102/88 cm)	26.0	19.4	22.7
Greece [139, 140]	2003-2004	4153	>18	ATP III [b]	24.1 24.2 [a]	22.9 22.8 [a]	23.6 [a]
Greece [141, 142]	2003-2004	9669	>18	ATP III [b]	24.8 [a]	24.2 [a]	24.5 24.5 [a]
				IDF			43.1 43.4 [a]
				JIS (102/88 cm)			26.3 [a]
				JIS (94/80 cm)			45.7 [a]
Greece [143]	2001-2002	3042	18-89	IDF	53.0	45.0	48.9
				JIS (102/88 cm)	25.0	14.9	20.0
Italy [144]	1997-1999	2100	≥ 19	ATP III	15.0	18.0	17.0
Italy [145]	2001	2388	> 25	ATP III	29.0	23.4	
Italy [146, 147]	2001	1929	40-79	ATP III [b]			22.5
Italy [148]	2001-2003	1564	45-64	ATP III			21.7
Italy [149]	2003-2004	345	>65	ATP III [b]	27.6	28.2	27.9

ATP III, National Cholesterol Education Program (NCEP) Adult Treatment Panel III; IDF, International Diabetes Federation; JIS, Joint Interim Statement; MetS, metabolic syndrome.

[a] Age-standardized prevalence of the MetS; [b] Modified classification.

Table 6. Metabolic syndrome crude prevalence – Northern Europe

Country	Study date	Sample size	Age range	MetS definition	Men (%)	Women (%)	Total (%)
	2000-2001	6105	30-79	IDF	44.7	39.1	
Finland [150]				JIS (102/88 cm)	36.9	34.5	
				JIS (94/80 cm)	47.8	40.7	
Finland [151]	2000-2001	6093	30-79	ATP III	30.2	31.2	30.8
Finland [152]	2004	3407	18-78	IDF			31.3
Sweden [153]	1991-1994	2064	45-69	ATP III			19.9
Sweden [154]	2002-2006	17544	45-64	ATP III			29.2
				IDF			45.2
				JIS (94/80 cm)			47.9
Norway [155]	1995-1997	10206	20-89	IDF	29.0	30.3	29.6
				JIS (102/88 cm)	26.8	25.0	25.9
Denmark [156]	2007-2008	15235	18-93	IDF [b]			27.0
Denmark [157]	2001-2003	5801	20-97	ATP III [b]	20.0	20.0	20.0
Denmark [158]	2003-2011	71527	20-100	JIS (94/80 cm) [b]	38.3	23.1	29.7
Estonia [159]	2008-2009	495	20-74	JIS (102/88 cm)	30.8 29.4 [a]	25.6 23.8 [a]	27.9 25.9 [a]

ATP III, National Cholesterol Education Program (NCEP) Adult Treatment Panel III; IDF, International Diabetes Federation; JIS, Joint Interim Statement; MetS, metabolic syndrome.

[a] Prevalence of MetS weighted for the average Estonian population (estimated in 2009); [b] Modified classification.

Table 7. Metabolic syndrome crude prevalence – Western Europe

Country	Study date	Sample size	Age range	MetS definition	Men (%)	Women (%)	Total (%)
France [170]	2006-2007	1856	18-74	ATP III [c]	14.4	13.7	14.1
				IDF	21.3	19.3	20.3
				JIS (94/80 cm)	22.8	19.4	21.1
				JIS (102/88 cm)	17.5	15.7	16.6
France [161]	2011-2012	7902	>18	JIS (94/80 cm)	18.9	9.5	12.2
Nederland [162]	2006-2012	59467	18-80	JIS (102/88 cm)	21.0	13.2	16.4
Nederland [163]	2008	1592	20-80	IDF			7.3
Nederland[164]	2006	14005	20-70	ATP III			15.5
Belgium [165]	2002-2004	992 [a]	18-75	IDF	12.7	8.5	
Luxembourg [166]	2007-2008	1349	18-69	JIS (94/80 cm)	35.5	20.4	28.0
				JIS (102/88 cm)	30.8	18.5	24.7
				IDF	31.0	19.3	
Switzerland [167]	2003-2006	4231	35-75	ATP III [c]	22.8	13.7	18.0
Germany [168]	2005	35869	18-99	ATP III	22.7 19.5 [b]	18.0 18.1 [b]	19.8 18.7 [b]
				IDF	40.3 34.1 [b]	28.0 27.7 [b]	32.7 30.7 [b]
				JIS (102/88 cm)	34.8 29.2 [b]	24.8 24.8 [b]	28.6 26.9 [b]
UnitedKingdom [169]	2003-2004	6073	> 48	ATPIII			13.6
United Kingdom [170]	1988-1990	1787	40-69	IDF			23.1
United Kingdom [171]	2002-2004	6810	45	JIS (102/88 cm) [c]	9.6	8.1	
Ireland [172]	2003-2005	1716	32-78	ATP III	15.8	9.3	13.2
				IDF	26.4	14.0	21.4

ATP III, National Cholesterol Education Program (NCEP) Adult Treatment Panel III; IDF, International Diabetes Federation; JIS, Joint Interim Statement; MetS, metabolic syndrome.

[a] Participants without CVD or diabetes; [b] Age-standardized prevalence according to the German population; [c] Modified classification.

Table 8. Metabolic syndrome crude prevalence – Eastern Europe

Country	Study date	Sample size	Age range	MetS definition	Men (%)	Women (%)	Total (%)
Croatia [173]	2008	2466	≥ 40	IDF			60.8
Croatia [174]	2002-2003	996	>18	ATP III [e]	28.0	39.0	34.0
Croatia [175]	2001	1394	≥20	ATPIII	28.3	23.2	25.0
				IDF	42.9	35.9	38.5
Slovakia [176]	2003-2005	1517	>18	ATP III	15.9	23.9	20.1
				IDF	39.7	36.6	38.1
Czech Rep[177]	2004-2006	805	18-65	IDF	32.5	22.9	26.4
Romania [178]	2005	1176 [a]	≥18	ATP III	38.3	42.3	40.6
				IDF	43.1	45.3	44.2
Romania [179]	Published 2009	1294	18-79	IDF	57.8	28.8	45.7
Romania [110]	2012-2014	2681	20-79	JIS (94/80 cm)	43.2	34.2	38.5
Bulgaria [180, 181]	2005-2008	575 [b]	19-98	ATPIII	23	23	23
				IDF	30	36	33
Bulgaria [182]	2005	2415	≥ 20	IDF	37.1	25.9	30.8
Hungary [183]	2006	1803	20-69	ATP III [e]	26.0	24.0	
				IDF	37.0	30.0	
				JIS (94/80 cm)	39.0 38.0 [c]	31.0 30.0 [c]	
Hungary [184]	2005	1762	20-69	IDF	39.3	33.9	36.4
Poland [185]	2003-2005	12567	20-74	IDF	30.7	26.8	28.6
				JIS (102/88 cm)	26.0	23.9	24.9
Poland [186]	Published 2010	1648	25-85	JIS (102/88 cm)	38.6	39.0	
				IDF	49.9	43.9	
Russia [187]	2000	3555	18-90	ATP III [e]	10.0	21.0	15.0
				IDF [e]	9.5	23.5	15.9
Turkey [188]	2003-2005	767	20-83	ATP III	23.1	33.5	28.8
				IDF	31.2	37.3	34.6
Turkey [189]	Published 2007	4259	≥20	ATP III [e]	28.0	39.6	33.9
Turkey [190]	2003	1568 [d]	≥20	JIS (102/88 cm)	41.0	43.0	38.0 [c]
				IDF	46.0	48.0	42.0 [c]

ATP III, National Cholesterol Education Program (NCEP) Adult Treatment Panel III; IDF, International Diabetes Federation; JIS, Joint Interim Statement; MetS, metabolic syndrome.

[a] Patients with CVD; [b] Subjects without personal or family history of CVD, hypertension or diabetes mellitus; [c] Age-adjusted prevalence of the MetS; [d] Non-diabetic participants; [e] Modified classification.

Table 9. Urban versus rural (or non-urban) metabolic syndrome crude prevalence

Country	Study date	Sample size	Age group	MetS definition	Rural (%)	Urban (%)	p-value
Spain [258]	2000-2003	809	35-74	JIS (102/88 cm) [a]	20.3	15.5	NS
Germany [259]	Published 2017	3863	18-65	ATPIII	M - 13.3 W - 6.3	M - 6.7 W - 6.1	<0.05 NS
USA [257]	1999-2006	6896	≥ 20	JIS (102/88 cm) [a]	T - 39.9 M - 39.7 W - 40.2	T - 32.8 M - 33.3 W - 32.3	<0.01 <0.05 <0.01
Palestine [260, 261]	1996-1998	992	30-65	WHO [a]	17.0	17.0	NS
United Arab Emirates [241]	1999-2000	4097	≥ 20	JIS (94/80 cm) [a]	T - 44.9 M - 37.3 W - 49.0	T - 38.2 M - 34.7 W - 41.0	0.010
				IDF	T - 44.3 M - 39.1 W - 47.3	T - 39.6 M - 36.8 W - 42.0	0.159
Chine [208]	2000-2001	15540	35-74	ATP III [a]	12.7	18.6	<0.05
India [252]	1991-1995	4044	35-64	ATP III	T - 11.1 M - 10.6 W - 11.6	T - 30.2 M - 26.8 W - 33.4	<0.001
Nigeria [253]	Published 2017	535	18-89	ATP III	13.7	28.2	<0.001
				IDF	12.2	30.8	<0.001
				JIS (94/80 cm)	12.2	33.3	<0.001
Cameroon [254]		1573	24-74	WHO	M – 1.9 W – 1.8	M - 7.3 W - 5.9	0.001 0.002
Ghana [255]	2002-2008	2220	18-99	ATP III [a]	M - 7.8 W - 11.2	M - 12.6 W - 21.4	NS <0.05
Thayland [223]	2008-2009	19256	≥ 20	JIS (90/80 cm)	M - 17.9 W - 27.7	M - 23.1 W - 24.5	<0.05 <0.05
Malasya [215]	2007-2011	8836	≥ 30	ATP III	28.5	25.0	0.001
				IDF	36.3	39.0	0.002
				JIS (90/80 cm)	42.5	44.6	0.018
Guatemala [263]	2002-2004	887	25-42	JIS (102/88 cm)	M (A) - 17.1 M (NA) - 23.5 W - 44.1	M - 27.7 W - 44.5	NS NS
Colombia [256]	1994-1996	615	≥ 30	WHO [a]	M - 2.8 W - 17.9	M - 14.1 W - 26.0	<0.01 NS
Brazil [262]	2013	435	≥ 60	ATP III [a]	M - 22.0 W - 37.0	M - 13.0 W - 40.0	NA NA

ATP III, National Cholesterol Education Program (NCEP) Adult Treatment Panel III; IDF, International Diabetes Federation; JIS, Joint Interim Statement; MetS, metabolic syndrome; T, total; M, men; W, women; A, agricultural; NA, non-agricultural; NS, not significant; NA, not available.

[a] Modified classification.

4. Metabolic syndrome associated risk: cardiovascular disease and type 2 diabetes

4.1. Physiopathology

The concept of IR was introduced in 1936 by Himsworth who suggested its relationship with diabetes mellitus [265]. Following the description in 1959, by Yalow and Berson, of an immunoassay technique for insulin, the theory of Himsworth was reinforced, by evidence of hyperinsulinism in individuals with hyperglycaemia [266]. In the 1960s, several authors [73, 267-270] reported associations between various risk factors for CVD that are now part of the metabolic syndrome. Even in the 1960s, associations of hyperinsulinism with CVD risk factors, other than hyperglycaemia, were reported [271-275]. In the 1970s and early 1980s, following the description of techniques to measure IR [276, 277], the concept of the association of several risk factors with each other [74, 76, 278-281] and with IR [75, 276, 279, 280, 282, 283] was reinforced.

With the introduction of "syndrome X" in 1988, by Reaven [12], IR was considered the key piece in the development of the syndrome. Although obesity was stressed as exacerbating factor, it was not recognized with the same pathophysiological significance of IR. In contrast, Kaplan, in 1989, included obesity in the "deadly quartet" [69]. Obesity would play a central role and IR would be a key intermediary in the pathogenesis of MetS. In the following years, the controversy was established regarding the relative importance of IR and obesity in the etiopathogenesis of MetS.

Being caused or not by obesity, IR may contribute through several mechanisms for the etiopathogenesis of the risk factors that integrate MetS as well as for the risk of CVD and type 2 diabetes [284-286].

The theory of lipotoxicity, based on the harmful effects of circulating free fatty acids (FFA) on insulin sensitivity [287-289], has been gaining consistency. FFAs are hydrophobic and thus circulate in the blood largely bound to albumin. The FFA originate mainly in the adipose tissue triglycerides deposits, through the action of three different hydrolases: adipose triglyceride lipase (ATGL or Pnpla2), hormone-sensitive lipase (Hsl) and monoglyceride lipase. Additionally, the FFA may also come from hydrolysis of triglyceride-rich lipoproteins in the vascular endothelium of peripheral tissues, mainly adipose and muscle tissue, through lipoprotein lipase (LPL) activity [290]. Insulin counteracts the release of FFA from adipose tissue and stimulates the LPL activity. Nevertheless, under IR conditions, the lipolytic effect in the adipose tissue, mainly related to ATGL hyperactivity [291] may predominate over LPL reduced action [292-294], and circulating FFA may increase.

The increase of lipolysis in adipose tissue is also associated with an increase in local inflammatory phenomena, reflected by an increase in macrophages and by the production of adipokines [295, 296]. According to the lipotoxicity model, increased circulating FFA may contribute to the aggravation of IR in insulin-sensitive tissues, namely in skeletal muscle and liver. However, FFA lipotoxicity can also manifest in other tissues with ectopic deposits of fat and contribute locally to the IR in these tissues.

Muscle tissue is the body's main site of insulin-mediated uptake of glucose. From the point of view of energy reserves, the skeletal muscle stands out due to its important glycogen and triglyceride deposits [297]. In states of muscular IR, the uptake of glucose is reduced, the production of glycogen is decreased, and the production of triglycerides is increased.

According to the glucose-fatty acid cycle, proposed by Randle [298], increased FFA muscular input may lead to a switch in fuel metabolism from carbohydrate to fat oxidation. FFA besides competing with glucose as the main source of energy in the muscle, may still contribute to hyperglycaemia by interfering with insulin mediated glucose uptake. FFA can cause muscle IR through interference with multiple signalling pathways [288, 299] by activating several protein kinases C (PKC) isoforms [300, 301], through the increase of intracellular levels of diacylglycerol (DAG) with subsequent suppression of tyrosine phosphorylation of the insulin receptor substrate [302], decreasing activation of phosphatidylinositol 3-kinase (PI3K), and thus reducing GLUT4 translocation, culminating in a reduction of muscle glucose uptake [303]. Moreover, other bioactive lipid metabolites such as long-chain fatty acyl CoA (the activated form of intracellular FFA) and ceramides [304] may interfere with normal insulin signalling and aggravate IR [305]. Glycogen synthesis, the non-oxidative glucose metabolism pathway, is activated by insulin. Under muscle IR conditions glycogen storage is compromised by decreased glucose uptake and decreased glycogen synthase activity [299]. In addition, the increased triglycerides content of the muscle in IR states, may result from a mismatch between the oxidation capacity and the increased availability of FFA [297].

As a consequence of IR, particularly in adipose tissue, the excessive supply of circulating FFA to the liver, may lead to an increase in intracellular FFA. The rate of FFA uptake from blood into hepatocytes depends on their concentration and intracellular transport capacity, namely through fatty acid transport proteins (FATP), as well as the ability to dissociate FFA from albumin [306]. As in muscle, increased hepatic FFA may interfere with insulin activation of the insulin receptor substrate through the activation of PKC isoforms and subsequent suppression of insulin receptor substrate tyrosine phosphorylation [307]. Under normal circumstances, the liver is the main source of endogenous glucose production. In the fasting state, where blood levels of insulin are usually low, the liver contributes to the maintenance of glucose homeostasis through increased glucose output. The hepatic output of glucose is ensured through the stimulation of glycogenolysis and gluconeogenesis. However, in an IR environment [308], the postprandial insulin-induced inhibition of gluconeogenesis and glycogenolysis, is compromised, leading to the endogenous production of glucose at the expense of gluconeogenesis and glycogenolysis, and as a result, hepatic output of glucose may increase [309]. Thus, in addition to adipose tissue, the liver also contributes to increased blood levels of glucose. On the other hand, the decrease in muscle uptake of glucose, due to IR, leads to a greater availability of glucose in the liver for the *de novo* lipogenesis [310] and consequent increase in the hepatic synthesis of triglycerides [311]. Hepatic *de novo* lipogenesis is a biosynthesis pathway of triglycerides from acetyl-CoA, which can be originated by several metabolic pathways, namely glycolysis. In addition to glucose, fructose is also a strong lipogenic substrate. The transcriptional regulation of *de novo* lipogenesis is mediated by the sterol regulatory element binding protein 1c (SREBP1c) and carbohydrate response

element binding protein (ChREBP). Hyperinsulinism, secondary to IR, may have a major contribution to the activation of *de novo* lipogenesis, through increased expression of hepatic SREBP1c which regulates the transcription of various enzymes involved in lipogenesis [312].

FFA, in addition to its action on IR, is still the major substrate for hepatic triglyceride synthesis [313, 314]. Although there are some manifestations of IR in the liver, lipogenesis continues to be stimulated by insulin [315, 316]. After entering into cells, FFA are rapidly activated by conversion to fatty acyl CoAs and then either oxidized or processed to triglycerides and stored.

FFA can be converted in the liver, through β oxidation and ketogenesis, in ketone bodies, an important energy source in the fasting state [317]. Increased liver glucose uptake and a possible decrease in AMP-activated protein kinase (AMPK) activity, an inhibitor of malonyl-CoA synthesis [318], may increase the intracellular levels of malonyl-CoA. Increased malonyl-CoA levels may lead to a decreased oxidation of fatty acids and to increased lipogenesis and intracellular DAG levels [319-322] which can activate PKC [323].

Increased hepatic triglyceride deposition leads to increased synthesis of apolipoprotein B-100 containing, very low-density lipoprotein (VLDL) [324] and subsequent increase in circulating VLDL and blood levels of triglycerides. The increase in circulating VLDL concentration associated with a normal activity of cholesteryl ester transfer protein (CETP) leads to an increase in triglyceride exchanges of VLDL by cholesterol of HDL. This process leads to a cholesterol-rich VLDL remnant particle that is atherogenic. On the other hand, the triglyceride-rich and low-cholesterol HDL

particles may undergo further hydrolysis of triglycerides and the dissociation of apo A-I protein. The free apo A-I in plasma is cleared more rapidly than apo A-I associated with HDL particles, leading to a reduced amount of circulating apo A-I and HDL particles, as well as lower HDL cholesterol levels.

Under physiological conditions, the FFA stimulate insulin secretion, namely in the basal status and potentiate glucose-stimulated insulin secretion [309]. Nevetheless, increased circulating FFA may be lipotoxic to beta cell and lead to a decrease in insulin secretion. Moreover, hyperglycaemia, resulting from decreased peripheral utilization and increased hepatic output, may contribute to decreased pancreatic insulin secretion through glycotoxicity.

In addition to the mechanisms proposed for the association of IR with hyperglycaemia, hypertriglyceridemia and low HDL cholesterol, IR and/or associated secondary hyperinsulinism may also contribute to BP elevation.

IR may contribute to the development of hypertension due to loss of the vasodilator effect of insulin [325] and pressor effect of FFA [326-328]. Likewise, secondary hyperinsulinism, due to IR, can promote hypertension [329-331] through several mechanisms that include: chronic enhancement of sympathetic nervous system activity, stimulation of the renin-angiotensin-aldosterone system (RAAS), modulation of cation transport and inducing vascular smooth muscle cell hypertrophy.

IR can also contribute to CVD risk by induction of a prothrombotic state and release of pro-inflammatory cytokines from the adipose tissue [332].

Adding to previously mentioned IR-related mechanisms, obesity itself may contribute to the etiopathogenesis of the various risk factors that integrate MetS. Obesity-related

IR is mainly due to the expansion of the adipose compartment and increased release of FFA [288, 333]. Subcutaneous adipose tissue (SAT) is an adipose compartment with lower cardiometabolic risk than VAT or other non-SAT locations, such as the liver and skeletal and cardiac muscle [334]. In fact, several studies have shown an association of non-SAT deposits, with a high risk for CVD [335] and diabetes [336]. The contribution of visceral fat deposits to IR is greater than that of subcutaneous fat, namely in women [337, 338]. The visceral lipolysis, according to the portal hypothesis, leads to an increased supply of FFA to the liver through the splanchnic circulation, which leads to greater liver exposure to FFA. In addition, VAT is also more metabolically active [336,339]. It is more sensitive to lipolysis and produces more inflammatory cytokines [340].

The association of adiposity, namely VAT, with hypertension is well documented [341, 342]. The retroperitoneal component of the VAT, which includes peri-renal fat, may also have a greater association with hypertension [343]. The association of obesity with hypertension may be explained by several mechanisms [337, 344, 345]. Reduced renal reabsorption of sodium and increase intravascular volume may be related to hyperinsulinism secondary to IR [346, 347]. Expanded adipose tissue, namely VAT and perivascular adipose tissue, may lead to the increased local production of angiotensinogen and activation of the RAAS [348-350]. Angiotensin II is also produced in adipose tissue not only by the usual route of RAAS but also by cathepsins and chymase [351]. In addition to adipose tissue synthesis, increased blood levels of angiotensinogen and angiotensin II have also been documented in obesity [352]. Adipocytes also produce aldosterone and increase vascular stiffness through vascular mineralocorticoid receptors [353]. Mineralocorticoid receptors are also present in adipose tissue and may

contribute to the synthesis of inflammatory adipokines and to the mediation of adipogenic actions of aldosterone and glucocorticoids [353, 354].

Sympathetic nervous system (SNS) [344, 355-361] activation, namely renal [362], may also play an important role in obesity [355, 356, 363, 364] Increased leptin [359, 365], secondary hyperinsulinism, impaired baroreflex sensitivity and brain RAAS may contribute to the enhanced SNS activity when obesity is present [366, 367].

Finally, obesity may influence several CVD risk factors through inflammation [368]. Adipokines are hormones produced in adipose tissue and may have multiple local and systemic actions. Adipokines produced in perivascular adipose tissue, primarily have local paracrine actions [369-371]. On the contrary, some of the adipokines produced at the usual adipose sites, namely in the VAT, can have a systemic contribution to the chronic inflammatory process. Some of the adipokines and cytokines produced locally may have pro-inflammatory actions (TNFα, IL-6, leptin, visfatin, resistin, adipocyte fatty-acid-binding protein, chemerin, C-reactive protein, retinol binding protein 4, SAA3, PAI-1) contributing to the adiposity-related, IR and/or to CVD or diabetes risk [36, 337, 372-374]. As contrast, others may have anti-inflammatory actions (omentin, apelin, adiponectin, fibroblast growth factor 21 and vaspin) and may be protective [368]. Obesity can trigger a positive imbalance between adipokines with harmful cardiovascular action and protective adipokines, thus leading to an increase in cardiovascular risk [375].

4.2. Cardiovascular disease risk

MetS is associated with an increased risk of CVD [376-380]. According to several meta-analyses [377, 378] the risk may even be higher for the WHO than for the ATP III definition. A systematic review including studies published from 1998 to 2005 [377], estimated a relative risk (RR) for CVD of 1.65 (95%CI: 1.38-1.99) and 1.93 (95%CI: 1.39-2.67), according to ATP III and WHO definitions, respectively. In addition, the RR for CVD may be higher in women compared with men [378]. MetS is also associated with an increased CVD mortality [378, 379, 381], namely in women. According to a systematic review and meta-analysis including prospective observational studies from 2002 to 2009 [381], the estimated RR for CVD mortality was 2.51 (95% CI: 1.38-4.55) and 1.93 (95% CI: 1.56-2.40), according to ATP III (2001) and AHA / NHLBI / ADA (2004), respectively. The MetS has also been associated with subclinical atherosclerotic disease, as several studies found significant associations between MetS and subclinical carotid damage, based on measurements of carotid intima-media thickness [382].

The MetS associated risk for CVD may be increased by the number of its individual components present. According to a USA study [383], the risk was significantly increased when 3 [OR 2.70 (95%CI:1.22-5.98)] and 4 or more [OR 5.86 (95%CI:2.51-13.66)] components were present.

Newer definitions such as IDF, AHA/NHLBI 2005 and JIS, lowered the thresholds for several of the MetS components and included treated individuals in each individual component, increasing this way the prevalence of the MetS compared with older

definitions, resulting in lower reported risks of CVD and associated mortality [192, 384-391].

The CVD risk associated with MetS may also be related to type 2 diabetes risk [392-394]. A meta-analysis of 102 prospective studies [393], found an increased risk for coronary heart disease (CHD) [hazard ratio (HR) 2.00 (95%CI:1.83-2.19)] in diabetic subjects. Even in individuals with no diabetes criteria, the presence of IFG increased the risk of CVD. According to a recent systematic review [395], impaired fasting glucose, defined by 100 mg/dL [396] or 110 mg/dL [397] cut-off points, increased the risk for CVD [RR 1.18 (95%CI:1.09-1.28) and RR 1.20 (95%CI:1.12-1.28) respectively], and the occurrence of diabetes [392] and IFG [394] may be related to the increased risk of CVD risk in women.

Hypertension may also play an important role in MetS-associated risk for CVD. In fact, high BP is a major cause of CVD risk [398-401] and mortality [402-406]. The CVD risk may even be higher in women [398]. The BP component of the MetS (≥ 130/85 mm Hg), in addition to its association with arterial stiffness [407] and carotid atherosclerosis [408], may also increase CVD risk [409-411]. In fact, the Framingham Offspring Study [409] showed a RR for CVD of 2.0 (95%CI:1.4-2.9) in participants with the BP component, compared with those who did not present the trait. Furthermore, the BP component was associated with CVD mortality [411, 412]. According to a Japanese study [412], the HR for CVD mortality was 2.07 (95%CI:1.21-3.52) in subjects with the blood component compared with those who did not present it.

Triglycerides and HDL cholesterol, the lipidic components of the MetS may also play an important role in the CVD risk. Triglycerides are inversely associated with HDL cholesterol [413], making it difficult to isolate each one`scardiovascular effects. In fact,

the strength of the association of triglycerides with CVD risk was greater if HDL was low [414, 415]. In contrast to triglycerides, low HDL cholesterol is a well-established independent risk factor for CVD [416]. The role of triglycerides on atherogenic risk was stressed by Lemieux with the use of the term "hypertriglyceridemic waist" [417]. Triglyceride levels are associated with increased CVD risk [418-427], namely in women [423]. This association has been supported by several meta-analyses [421, 423, 426]. The strength of the association may be greater in subjects with impaired glucose tolerance or type 2 diabetes [428, 429]. Randomized controlled trials suggested a stronger association of glucose, HDL cholesterol and BP components of MetS with CVD risk, over triglycerides trait [386, 430-433]. According to these data, the triglycerides component may play an important role as a biomarker rather than as a risk factor for CVD. However, in the NHANES study, the triglycerides MetS component had a stronger association with CHD risk [OR 1.66 (95%CI:1.20-2.30), than the other components, namely HDL component [OR 1.35 (95%CI:105-1.74)] [434].

Obesity is independently associated to CVD risk [21, 22]. In addition, AO may even be a better risk predictor than total body fat [28]. In the Botnia study [411], the adiposity component of MetS (according to WHO definition) was significantly associated with CVD risk [RR 1.44 (p=0.07)], but dyslipidemia and BP components showed stronger associations. The Framingham Offspring Study [409] also showed an increased risk of CVD [RR 1.9 (95%CI:1.4-2.5)] for AO (according to ATP III definition) but weaker than for the glucose and BP components. On the other hand, a study of elderly Danish women [410] reported a significant association of CVD risk only with the AO (central/peripheral fat mass rate > 1 or BMI >30 Kg/m^2) and BP components [HR1.48 (95%CI: 1.30, 1.48) and HR 1.19 (95%CI: 1.09, 1.30), respectively].

4.3. Type 2 diabetes risk

MetS is a risk factor for type 2 diabetes [377, 435, 436]. According to a meta-analysis, including prospective observational studies from 1998 to 2008 [436], the RR for diabetes was 5.17 (95%CI;3.99-6.69), 4.45 (95%CI:2.41-8.22), 3.53 (2.84-4.39), 5.12 (95%CI:3.26-8.05) and 4.42 (95%CI:3.30-5.92), for WHO, EGIR, ATP III, AHA/NHLBI 2005 and IDF definitions, respectively.

As observed for CVD risk, the risk of type 2 diabetes may be higher with increasing number of components present [383, 409, 437]. An USA study [383], found a significant increased risk for the presence of 2 [OR 5.99 (95%CI:1.44-24.98)], 3 [OR 9.37 (95%CI: 2.22-39.59)] and 4 or more [OR 33.67 (95%CI:7.93-142.96)] components. In addition, the risk by number of MetS traits, according to glucose component status, is greater for all 4 possible traits grouping when this component is present [437]. In fact, according to the Framingham Offspring Study [409] the glucose component alone, has more predictive value for type 2 diabetes risk [RR 12.5 95%CI:9.1-17.3)] than any trait grouping of 3 of the other 4 components [(RR 5.0 (95%CI:3.7-6.8)] of MetS.

According to the Framingham Offspring Study [409], although the BP component increased the risk of diabetes [RR 2.4 (95%CI:1.7-3.5)], the magnitude of the association was lower than that for the other MetS components (RR between 2.7 and 12.5). However, a Japanese study [437] showed that the BP component was in fact associated with diabetes risk even in normoglycemic subjects [HR 1.8 (95%CI:1.4-2.5)]; in addition, this association increased substantially in subjects with impaired fasting glucose [HR 17.4 (95%CI:14.2-21.3)].

Triglycerides levels are often high in type 2 diabetes [438]. Furthermore, triglycerides levels may increase the risk of diabetes [439-441]. According to a recent study [442] the risk of diabetes and impaired fasting glucose was increased by 4% and 2%, respectively, for every 10 mg/dL elevation of the triglycerides levels. Nevertheless, Mendelian randomization studies [443] suggested that triglycerides may soley be a biomarker and not a causal factor.

HDL cholesterol levels are also associated with increased risk of diabetes in epidemiological studies [444-445]. However, as for triglycerides, Mendelian randomization studies do not support a causal role for HDL cholesterol [446].

Obesity is independently related to type 2 diabetes [29]. Excluding the glucose component, the adiposity one appears to be more strongly associated with diabetes risk than other components [383, 409, 410, 437]. The Framingham Offspring Study [409] showed a RR for diabetes of 4.1 (95%CI: 3.0, 5.6) in subjects with the AO component, compared with individuals without this trait, being only exceeded by the glucose component [RR 12.5 (95%CI: 9.1, 17.3)]. In addition, according to a study of elderly Danish women [410], AO and glucose components were the only ones that were associated with type 2 diabetes [HR1.98 (95%CI: 1.57, 2.48) and HR 3.38 (95%CI: 2.71, 4.22), respectively].

5. The metabolic syndrome: endocrine disorders

The endocrine system has a main role in the energetic balance, BP and glucose and lipid levels [447-452]. Additionally, insulin sensitivity is dependent on complex endocrine regulation mechanisms. Thus, from this point of view, the MetS itself can be envisioned as an endocrine disease.

Several rare endocrine disorders have been related to MetS, CVD and diabetes risk, namely acromegaly and Cushing syndrome. Increased production of growth hormone in acromegaly is associated with IR [453, 454], dyslipidaemia [455], hypertension [455], CVD [455, 456] and diabetes risk [457-459]. Cushing syndrome is associated with AO [460], MetS [461-464], dyslipidemia, hypertension and increased risk of CHD [465-467] and diabetes [468].

Among the endocrine diseases that have been associated with the MetS, thyroid diseases and hypovitaminosis D are highlighted due to their high prevalence.

Although several studies have reported an association of MetS with hypovitaminosis D and thyroid dysfunction, namely hypothyroidism, the evidence is scarcer as far as thyroid autoimmunity is concerned. In Portugal, apart from the fact that no study has yet been published on the subject of MetS and its possible associations with 25(OH)D, thyroid-stimulating hormone (TSH), thyroid hormones and thyroid antibodies serum levels, there is little data available on hypovitaminosis D, thyroid dysfunction and thyroid autoimmunity. Thus, the approach of this specific endocrine pathology, in the context of the investigation of MetS in Portugal, aims to contribute not only to the clarification of its association with cardiometabolic risk but also to a better characterization of its national prevalence.

5.1. Vitamin D and the Metabolic Syndrome

Vitamin D (VitD), is actually a fat-soluble hormone with multiple actions, notably those involving calcium homeostasis and bone metabolism [469]. Its cellular action is exerted through the binding to the nuclear VitD receptor (VDR), expressed in the majority of cells and with important effects on the regulation of the transcription of multiple genes. 1alpha, 25-dihydroxyvitamin D (1α,25(OH)^{2}D) or calcitriol, the physiologically active form of VitD is produced from two prohormones, cholecalciferol (VitD3) and ergocalciferol (VitD2). VitD3, the main source of VitD, originates mainly in the skin where it is synthesized through photolytic conversion, from the cholesterol precursor 7-dehydrocholesterol, through exposure to ultraviolet (UV) B radiation (290-315 nm). A small part also comes from the diet. On the other hand, VitD2 comes exclusively from dietary sources. These forms of VitD are not biologically active and must undergo two hydroxylations to be active. The first step in VitD activation occurs in the liver through a 25-hydroxylation, with the formation of 25-hydroxyvitamin D [25(OH)D], also known as calcidiol, which has low bioactivity but is the main form of VitD in the blood stream and the best indicator of VitD status. In a second activation step, 25(OH)D undergoes a 1α-hydroxylation, primarily in the kidney, leading to the synthesis of the active 1α,25(OH)^{2}D.

There is general agreement that the lower limit of an adequate VitD status corresponds to a serum 25 (OH) D value of 10-12 ng/mL (or 25-30 nmol/L) [470]. Levels greater than or equal to 10-12 ng/mL have a beneficial effect on bone health by preventing rickets and osteomalacia. According to the Institute of Medicine (IOM) recommendations [471], serum levels of 25(OH)D between 12 and 20 ng/mL (or between 30 and 50 nmol/L)

are “inadequate” in some people. In addition, serum levels >20 ng/ml (or 50 nmol/L) are “sufficient” for almost the whole population. Nevertheless, there is still an intense discussion about optimal 25(OH)D values to not only maintain bone health but also other body systems influenced by VitD [472].

Studies conducted in Portugal, to assess the prevalence of hypovitaminosis D had a regional scope and in most cases involved specific groups recruited in a hospital context.

According to a study performed in a hospital in the North of Portugal [473], which included 5439 25(OH)D assays, a median of 17 ng/mL was found for 25(OH)D values; in addition, values ≤ 20 ng/mL were found in 60.3% of the cases. Another study performed in the Center of Portugal [474], including 123 hospitalized patients, showed a prevalence of 25(OH)D values less than 10 ng/mL or less than or equal to 20 ng/mL of 67.5% and 92.7%, respectively. Furthermore, another study also performed at a hospital in Center of Portugal [475], including 2071 25(OH)D assays, found a mean value of 11 ng/mL and a prevalence of 65% for 25(OH)D values below 20 ng/mL. A study conducted with 198 healthy volunteers aged 18-67 years in the city of Porto [476] showed mean levels of 22.2 ng/mL (or 55.4 nmol/L) for 25(OH)D; additionally, 48% of the participants presented levels below 20 ng/mL (74% in the winter period). According to a recent study of the EPITeen project [477], including 514 adolescents from the city of Porto, the mean value of 25(OH)D was 16.5 ng/mL.

According to a systematic review on the worldwide prevalence of hypovitaminosis D [478], that did not included Portugal, there was a large dispersion of mean and median values estimates for 25(OH)D (4.9 to 136.2 nmol/L and 20.7 to 91.0 nmol/L,

respectively). In addition, 37.3 % and 6.7% of the samples included in this review presented mean levels below 20 ng/mL and 10 ng/mL, respectively. Taking these values into account, hypovitaminosis D gains a worldwide dimension [470, 478].

In addition to the effects on bone health, an inadequate vitD status has been associated with the CVD risk [479-490]. Hypovitaminosis D is also associated with subclinical atherosclerosis [491, 492]. This association with the CVD risk may be related to the role of VitD on cardiovascular physiology [481], through several mechanisms that include, a negative regulation of the RAAS, direct effect on calcium flow and cardiac myocyte growth, regulation of vascular calcification, maintenance of endothelial function, and anti-inflammatory and immunomodulatory actions.

An inadequate VitD status has been associated with hypertension [493-496]. Hypovitaminosis D has also been associated with type 2 diabetes risk [497-499]; several meta-analyses also supported this association [500-504]. Additionaly, a negative association of 25(OH)D levels with all-cause and CVD mortality has been also suggested [505-508].

According to several meta-analyses a negative association was found between 25(OH)D levels and MetS [502, 509]. This association has been documented in both cross-sectional studies [171, 510-516] and in prospective studies [517-521].A recent meta-analysis [509], including 16 cross-sectional studies, found a MetS OR of 0.87 (95% CI 0.83, 0.92) for a 25 nmol/L increment in blood 25(OH)D levels.

The association of 25(OH)D levels with WC [510, 513, 516] and its MetS component [171, 511, 512, 514, 515] is well documented. Also, several studies reported an association with triglycerides levels [513, 516] and their respective MetS component [171, 511, 512, 514, 515]. The

associations of 25(OH)D with BP [510, 513, 516] and its MetS component [171, 512, 514, 515] as well as with glycemia [513, 516] and its component [171, 511, 512, 515] have also been reported. Less frequently the association with HDL cholesterol [510, 513, 516] and its component [171, 515] has been found.

In addition, 25(OH)D level are positively associated with insulin sensitivity [522] and hypovitaminosis may have a negative impact on beta cell function [523].

The association of parathyroid hormone (PTH), a hormone with a close regulatory relationship with VitD, with CVD risk, has been the subject of some controversy [524, 525]. A meta-analysis on the subject, including 12 studies [526] reported a positive association of PTH serum level with total CVD events (pooled HR 1.45; 95% CI 1.24, 1.71). However, the large Atherosclerosis Risk in Communities Study did not show an association with any CVD outcome [527].

According to a recent meta-analysis [528] including 10 studies, which evaluated the relationship between PTH serum levels and all-cause and CVD mortality, there was an increased all-cause mortality (pooled RR 1.19; 95%CI 1.08, 1.30) in both sexes and an increased CVD mortality (pooled RR 1.68; 95% CI 1.05, 2.67) only in men.

Findings from population-based cross-sectional studies are not concordant [525], with some studies finding a positive association between PTH serum levels and MetS [529-532] while in others no association was shown [512, 514, 519, 533-535]. This association was more consistent in elderly men [510], morbidly obese individuals [530] and primary hiperparathyroidism [531, 532]. The association of PTH with MetS may be explained mainly by the associations with the WC [510, 530], BP [536], glycaemia and HDL cholesterol risk factors included in the MetS definition [525].

5.2. Thyroid function and autoimmunity

The thyroid is an important endocrine organ responsible for the production of thyroid hormones, triiodothyronine (T3) and thyroxine (T4), both having extensive metabolic actions. Thyroid dysfunction is a frequent pathology and may take two forms, hypothyroidism and hyperthyroidism, signifying a decrease or increase hormone production, respectively. Within each type of dysfunction, we can still consider overt and subclinical forms [537].

In Europe, and according to a recent meta-analysis [537], the prevalence of thyroid dysfunction was 3.82%. In addition, the prevalence of hypothyroidism and hyperthyroidism was 3.05% and 0.75%, respectively. According to the same study, subclinical forms of thyroid dysfunction predominated (85.2%) compared to overt forms. Moreover, a high prevalence of undiagnosed dysfunction (4.94% and 1.72% for hypothyroidism and hyperthyroidism, respectively) was found.

The information available in Portugal on thyroid dysfunction is scarce, namely with regard to the national prevalence of hypothyroidism and hyperthyroidism.

Iodine deficiency is the leading cause of thyroid disorders, namely goiter and hypothyroidism in regions of the globe with inadequate intake of iodine [538]. In regions with sufficient iodine intake, thyroid autoimmune disorders play a greater role in the etiology of thyroid dysfunction. Among thyroid autoimmune disorders, Hashimoto thyroiditis, also known as chronic autoimmune thyroiditis (AIT), stands out for its higher prevalence. AIT is still the main cause of hypothyroidism in iodine-sufficient areas [538]. AIT, as the name implies, results from a chronic inflammatory process involving autoimmune mechanisms that translate into lymphocytic infiltration and

fibrosis of the gland and the presence of thyroglobulin antibodies (TgAb) and/or thyroid peroxidase antibodies (TPOAb) in the blood of most patients [539]. As for thyroid dysfunction, AIT is poorly studied in Portugal.

Thyroid hormones have important effects on the cardiovascular system [540] and CVD risk, and mortality may be increased in hypothyroidism [541, 542]. Low and low-normal thyroid function [543] may also increase the risk of type 2 diabetes and excess thyroid hormones increase glycaemia [544].

According to several cross-sectional studies, TSH and thyroid hormones may have an association with the prevalence of MetS and its components. Moreover, and although the mechanisms remain unclear, the contribution of IR has been consistently reported [545]. A positive association of TSH [546-549] and FT3 [550, 551] with IR was documented. In contrast, a negative association was reported for FT4 [547, 548, 552].

Several cross-sectional studies conducted in euthyroid individuals [546-550, 552-562], have consistently shown a positive association of TSH serum levels with MetS (Table 10). In addition, a positive association with the triglycerides component has been increasingly reported. Nevertheless, the associations with BP, HDL cholesterol, glucose and WC components are less clear.

Regarding FT4 (Table 11), several studies [547, 548, 550, 552-556, 559-563] have documented a negative association with the MetS and its triglycerides and WC components. Furthermore, a positive association of FT3 with MetS and its BP, glucose, triglycerides and WC components has been reported (Table 12) [550, 551, 553, 554, 559, 560, 562, 564].

These findings can be explained by the diversity of thyroid hormone effects on energy homeostasis, lipid and glucose metabolism and BP [565, 566].

The role of AIT in CVD and MetS risk is not yet well defined. AIT may act as an independent CVD risk factor through the promotion of chronic inflammation [567, 568]. According to a recent study [567], the risk for coronary heart disease was increased in AIT (HR of 1.44; 95%CI 1.05-1.99). Other studies also found an association with CHD [568] and stroke [569]. Additionaly, a cluster analysis [570] found an association between increased TPOAb and homocysteine levels suggesting an adverse effect of these alterations in left ventricular systolic function. However, an individual participant data analysis of subjects with subclinical hypothyroidism [571], did not show an increased CHD risk associated with the presence of TPOAb, suggesting that the cardiovascular effects of thyroid autoimmunity can be mediated by thyroid dysfunction. Moreover, a 20-year follow up study, of the original Whickham study showed no association of AIT with coronary heart disease [572]. Regarding possible associations between AIT and MetS the information is very scarce. In fact, only one study conducted in postmenopausal euthyroid women [573] was published on the subject, and no association was observed between TgAb and TPOAb levels and MetS.

Table 10. Associations between TSH and Metabolic Syndrome and its components

Country	Study date	Sample size	Age group	MetS	IR	BP	Gly	HDL	TG	WC
Roos, 2007 [553]	1998-2003	1581 [a]	28-75	-	No [h]	No [h]	No [h]	No [h]	Yes (+) [h]	No [h]
Pergola, 2008 [554]	Published 2008	651 [p]	18-68	No [h]	-	No*	No*	No*	No*	No*
Park, 2009 [555]	2006-2007	949 [b]	PM	Yes (+) [h]	No	DBP (+)*	No *	No *	Yes (+) *	No *
Garduno-Garcia, 2010 [552]	Published 2010	2771 [c]	18-70	-	No	No *	No *	No *	Yes (+) *	No *
Ruhla, 2010 [546]	Published 2010	1333 [c]	> 18	Yes (+) [h]	Yes (-)	No*	No*	No*	Yes (+) *	Yes (+) * [o]
Park, 2011 [556]	2002-2009	5998 [c]	> 18 [d]	Yes (+) [i]	No	No	No	Yes (-)	Yes (+)	Yes (-)
Tarcin, 2012 [550]	Published 2012	211	18-73 [e]	No (+) [j]	-	No	No	No	No	No
Waring, 2012 [557]	1997-1998	1779 [c]	70-79	Yes (+) [k]	-	-	-	-	-	-
Oh, 2013 [558]	2008-2010	2760 [b]	18-39	Yes (+) [l]	No	Yes (+)	No	No	Yes (+)	Yes (+)
Roef, 2014 [559]	2002-2004	2315 [c]	35-55	No [l]	-	Yes (+)*	No*	No*	Yes (+)*	No*
Mehran, 2014 [547]	1997-2004	3755 [c]	≥ 20	No [i]	Yes (+)	No	No	No	Yes (+)	No
Laclaustra, 2015 [548]	Published 2015	3533 [b]	20-65 [f]	Yes (+) [n]	Yes (+)	Yes (+)	No	No	Yes (+)	No
Kim, 2016 [560]	2006	13,496	35-65 [g]	No [i]	-	No	Yes (-)	Yes (+)	No	Yes (-) [o]
Park, 2017 [549]	2007-2014	132,346 [b]	>18	Yes (+) [i]	Yes (+)	SBP (+)*	Yes (+)*	Yes (-)*	Yes (+)*	Yes (+)*
Mehran, 2017 [561]	1997-2004	5,422 [c]	≥20	- [i]	No	No*	No*	No*	Yes (+)*	No*
Wolffenbuttel, 2017 [562]	2007	26,719 [b]	18-80	No [n]	-	No	W:Yes (+)	M:Yes (-)	Yes (+)	No

[a] Age and gender adjusted; [b] adjusted for age and other variables (excluding gender); [c] adjusted for age, gender and other variables; [d] included suclinical forms; [e] only obese participants; [f] only men; [g] total T3 and T4 levels, [h] ATP III (2001); [i] JIS modified; [j] AHA/NHLBI (2005); [k] ATP III modified; [l] IDF; [m] specific Japanese definition [504]; [n] JIS; [o] WC replaced by BMI; [p] BMI≥ Kg/m^2.

W, women (only); M, men (only); PM, postmenopausal women; MetS, metabolic syndrome; IR, insulin resistance; BP, Blood pressure; Gly, Glycaemia; HDL, HDL cholesterol; TG, triglycerides; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 11. Associations between FT4 and Metabolic Syndrome and its components

Study	Study date	Sample size	Age group	MetS	IR	BP	Gly	HDL	TG	WC
Roos, 2007 [553]	1998-2003	1581 [b]	28-75	- [i]	Yes (+)	No	Yes (-)	Yes (+)	Yes (-)	Yes (-)
Pergola, 2008 [554]	Published 2008	651 [p]	18-68	No [i]	-	No*	No*	No*	No*	No*
Park, 2009 [555]	2006-2007	949 [c]	PM	No [i]						
Kim, 2009 [563]	2005-2006	44, 196 [a]	27-70	No [k]	-	Yes (+)	Yes (+)	Yes (-)	Yes (+)	Yes (-)
Garduno-Garcia, 2010 [552]	Published 2010	2771 [d]	18-70	- [i]	Yes (-)	No *	No *	Yes (+) *	No *	No *
Park, 2011 [556]	2002-2009	5998 [d]	> 18 [e]	No [j]	No	DBP (+)	No	Yes (+)	Yes (-)	Yes (+)
Tarcin, 2012 [550]	Published 2012	211 [a]	18-73 [f]	Yes (+) [k]	No	Yes (+)	Yes (+)	No	No	No
Roef, 2014 [559]	2002-2004	2315 [d]	35-55	Yes (-) [m]	-	No*	No*	No*	Yes (-)*	Yes (-)*
Mehran, 2014 [547]	1997-2004	3755 [d]	≥ 20	Yes (-) [j]	Yes (-)	Yes (+)	No	Yes (+)	Yes (-)	Yes (-)
Laclaustra, 2015 [548]	Published 2015	3533 [c]	20-65 [g]	Yes (-) [o]	Yes (-)	No	No	No	Yes (-)	No
Kim, 2016 [560]	2006	13,496	35-65 [h]	No [j]	-	Yes (+)	No	No	No	No [p]
Mehran, 2017 [561]	1997-2004	5,422 [d]	≥20	- [j]	No	DBP (-)	Yes (+)	No	Yes (-)	Yes (-)
Wolffenbuttel, 2017 [562]	2007	26719 [c]	18-80	Yes (-) [o]	-	W:SBP (+)	M:Yes (-)	M:Yes (+)	Yes (-)	Yes (-)

* Continuos variables (not MetS components).

[a] Age adjusted ; [b] Age and gender adjusted; [c] adjusted for age and other variables (excluding gender); [d] adjusted for age, gender and other variables; [e] included suclinical forms; [f] only obese participants; [g] only men; [h] total T3 and T4 levels, [i] ATP III (2001); [j] JIS modified; [k] AHA/NHLBI (2005); [l] ATP III modified; [m] IDF; [n] specific Japanese definition [504]; [o] JIS; [p] WC replaced by BMI; [q] BMI≥ Kg/m^2.

W, women (only); M, men (only); PM, postmenopausal women; MetS, metabolic syndrome; IR, insulin resistance; BP, Blood pressure; Gly, Glycaemia; HDL, HDL cholesterol; TG, triglycerides; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Table 12. Associations between FT3 and Metabolic Syndrome and its components

Study	Study date	Sample size	Age group	MetS	IR	BP	Gly	HDL	TG	WC
Roos, 2007 [553]	1998-2003	1581 [a]	28-75	-	No	No	No	No	Yes (-) [g]	No
Pergola, 2008 [554]	Published 2008	651 [m]	18-68	No [g]	-	No*	No*	No*	No*	No*
Tarcin, 2012 [550]	Published 2012	211	18-73 [e]	No [h]	No	No	No	No	No	No
Roef, 2014 [559]	2002-2004	2315 [c]	35-55 [n]	Yes (+) [n]	-	Yes (+)*	No*	Yes (-)*	Yes (+)*	Yes (+)*
Kim, 2016 [560]	2006	13,496	35-65 [f]	Yes (+) [j]	-	Yes (+)	Yes (+)	Yes (-)	Yes (+)	Yes (+) [l]
Kim, 2017 [551]	2006-2012	12,037	35-65	Yes (+)	Yes (+)	Yes (+)*	Gli (+)*	Yes (+)*	Yes (+)*	-
Ferrannini, 2017 [564]	2002-2004	940	30-60	-	Yes (+)	Yes (+)*	No*	Yes (-)*	Yes (+)*	-
Wolffenbuttel, 2017 [562]	2007	26719 [b]	18-80	Yes (+) [k]	-	Yes (+)	Yes (+)	Yes (-)	Yes (+)	Yes (+)

* Continuos variables (not MetS components).

[a]age and gender adjusted; [b] adjusted for age and other variables (excluding gender); [c] adjusted for age, gender and other variables; [d] included suclinical forms; [e] only obese participants; [f] total T3 and T4 levels, [g] ATP III (2001); [h] AHA/NHLBI (2005); [i] specific Japanese definition [504]; [j] JIS modified; [k] JIS; [l] WC replaced by BMI; [m] BMI≥ Kg/m^2; [n] IDF.

MetS, metabolic syndrome; IR, insulin resistance; BP, Blood pressure; Gly, Glycaemia; HDL, HDL cholesterol; TG, triglycerides; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure.

II. Objectives

The main objective of this thesis was to evaluate the prevalence of MetS and its determinants in Portugal, having a specific focus on the influence of obesity and endocrine pathology, namely hypovitaminosis D and thyroid dysfunction and autoimmunity. In order to carry out this main objective, several specific objectives were set:

1. To assess which adiposity measure better perform in the identification of MetS in a sample of Portuguese adults and to estimate the cut-off values for these adiposity anthropometric measures (Paper 1).
2. To assess the prevalence of MetS and its determinants in the overall and administrative regions of the Portuguese mainland, according to the 2009 JIS definition, using the WC cut-off points that that best fitted the Portuguese population (Paper 2).
3. To evaluate the prevalence of hypovitaminosis D and its determinants as well as PTH serum levels determinants and associations of the 25(OH)D and PTH serum levels with MetS and its individual components in a sample of the Portuguese mainland population (Paper 3).
4. To evaluate in a sample of the Portuguese population, the prevalence of thyroid dysfunction and antibody positivity and to assess the associations of thyroid-stimulating hormone (TSH), thyroid hormones and thyroid antibodies with the MetS, its components (Paper 4).

III. General Methodology

1. PORMETS study

PORMETS is a national cross-sectional study. A sample of adults registered at primary health care centres throughout mainland Portugal was selected. In each of the eighteen Portuguese mainland administrative regions (districts), two health care centres were included, one in the district's capital and the other representative of a non-urban area. Apart from the district of Setubal that only included one centre, all the others included two, for a total of 35 health care centres.

1.1. Participants

At each health care centre, participants were randomly selected from the general practitioner's patient lists, and 120 participants were recruited, with an inclusion criterion of being aged 18 or older. A total of 4105 participants was evaluated, and information was collected from February 2007 to July 2009. Ten participants were excluded from the data analysis because they were pregnant at the time of the interview, resulting in 4095 remaining participants.

After excluding participants who had missing information on the MetS features, for all the three operational definitions (the ATP III 4, 5, IDF 10, 11, and JIS 1) considered, the analysis to assess the prevalence of MetS and its determinants in the overall and administrative regions of the Portuguese mainland, included 4004 participants, 2309 women and 1695 men.

After additional exclusion of participants with missing information on height, weight, WC, and HC, the remaining 3,956 participants (2,287 women and 1,669 men) were included in the data analysis to assess which adiposity measure performed better in identifying MetS and to estimate the cut-off values for these anthropometric measures.

A sub-sample including 500 participants (286 women and 214 men) was randomly selected from the initial PORMETS sample. This sub-sample size was calculated considering a margin of error of 5%, confidence level of 95% and response distribution of 50% for the proportion of participants with MetS, 25(OH)D levels below 30 ng/mL (75 nmol/L), thyroid dysfunction and thyroid antibody positivity. The sub-sample was used to evaluate the prevalence of hypovitaminosis D and its determinants as well as PTH serum levels determinants and associations of the 25(OH)D and PTH serum levels with MetS and its individual components. The comparison between the selected (500) and non-selected participants (3595) did not show significant differences, except for systolic BP ($p=0.038$) and for insulin serum levels ($p=0.040$), although, the magnitude of the differences between estimates were low (Table 13).

This sub-sample of 500 participants, was also used to evaluate the prevalence of thyroid dysfunction and antibody positivity and to assess the associations of TSH, thyroid hormones and thyroid antibodies with the MetS, its components, and other possible determinants. After exclusion of 14 participants with missing values for TSH, 486 participants (281 women and 205 men) remained. Participants with previously diagnosed hypothyroidism (n=7) and under treatment with L-thyroxine were included in the evaluation of the prevalence of thyroid dysfunction but excluded for the remaining analyzes, to minimize the influence of medication in the results. The

comparison between the selected (486) and non-selected (3595) participants did not show significant differences ($p<0.05$), except for insulin serum levels ($p=0.046$), but again without relevant differences between estimates.

Table 13. Comparison between the 500 randomly selected and non-selected participants

Variables		Non-selected participants	Selected participants	p value
Gender [(n (%)]	Women	2069 (57.6)	286 (57.2)	
	Men	1526 (42.4)	214 (42.8)	0.881
Age (years)	[median (P25, P75)]	54 (41, 66)	53 (41, 67)	0.856
Education level (years)	[median (P25, P75)]	4 (4, 9)	6 (4, 9)	0.224
Alcohol intake [(n (%)]	No	1621 (45.2)	229 (45.9)	
	Yes	1969 (54.8)	270 (54.1)	0.756
Smoking habits [(n (%)]	No	2487 (69.3)	359 (71.8)	
	Yes	1104 (30.7)	141 (28.2)	0.247
Physical exercise [(n (%)]	No	2592 (73.1)	352 (71.7)	
	Yes	954 (26.9)	139 (28.3)	0.511
UV exposure [(n (%)]	Lower	1971 (55.9)	283 (56.6)	
	Higher	1555 (44.1)	217 (43.4)	0.768
Weight (Kg)	[median (P25, P75)]	71.0 (62.2, 80.8)	71.0 (61.0, 80.5)	0.704
Height (cm)	[median (P25, P75)]	162.0 (156.0, 169.0)	162.0 (155.0, 169.0)	0.745
BMI (Kg/m^2)	[median (P25, P75)]	27.0 (24.0, 30.0)	27.1 (24.1, 29.8)	0.813
WC (cm)	[median (P25, P75)]	93.5 (85.0, 101.5)	94.0 (86.0, 102.0)	0.399
Systolic BP (mmHg)	[median (P25, P75)]	130 (117, 145)	131 (119, 147)	0.038
Diastolic BP (mmHg)	[median (P25, P75)]	79 (70, 86)	80 (70, 87)	0.073
Glucose (mg/dL)	[median (P25, P75)]	85 (77, 97)	85 (77, 97)	0.947
Insulin (µU/mL)	[median (P25, P75)]	7.6 (5.1, 11.4)	8.1 (5.3, 12.2)	0.040
HOMA	[median (P25, P75)]	1.6 (1.0, 2.6)	1.7 (1.1, 2.9)	0.119
hs-CRP (mg/L)	[median (P25, P75)]	0.15 (0.07, 0.36)	0.16 (0.08, 0.39)	0.626
Cholesterol (mg/dL)	[median (P25, P75)]	206 (179, 234)	205 (180, 233)	0.611
Triglycerides (mg/dL)	[median (P25, P75)]	106 (78, 147)	104 (76, 143)	0.242
HDL cholesterol (mg/dL)	[median (P25, P75)]	47 (39, 55)	47 (39, 55)	0.553
MetS [(n (%)]	No	2086 (59.8)	307 (61.6)	
	Yes	1403 (40.2)	191 (38.4)	0.428
BP component [(n (%)]	No	1352 (38.4)	177 (35.5)	
	Yes	2172 (61.6)	322 (64.5)	0.212
WC component [(n (%)]	No	1812 (51.6)	253 (50.6)	
	Yes	1702 (48.4)	247 (49.4)	0.686
Glucose component [(n (%)]	No	2655 (76.4)	382 (77.3)	
	Yes	822 (23.6)	112 (22.7)	0.635
HDL-C component [(n (%)]	No	1566 (44.7)	224 (44.8)	
	Yes	1935 (55.3)	276 (55.2)	0.977
TG component [(n (%)]	No	2615 (74.9)	383 (76.6)	
	Yes	878 (25.1)	117 (23.4)	0.401

UV, ultraviolet; BMI, body mass index; WC, waist circumference; BP, blood pressure; HOMA, homeostatic model assessment; hs-CRP, high sensitivity C-reactive protein; HDL, high density lipoprotein; MetS, metabolic syndrome; TG, triglycerides.

1.2. Methods

All of the Portuguese Regional Health Administrations, the Ethics Committee of the São João Hospital E.P.E. (authorized in 27th February 2007) and the Portuguese Data Protection Authority (authorization number: CNPD 1053/2007) approved PORMETS. The coordinator of each health care centre also provided authorization and all participants provided written informed consent.

A trained nurse administered a structured questionnaire with only closed-ended questions; information was collected regarding personal medical history and socio-demographic and behavioural characteristics. A participant was considered a current smoker if he/she smoked daily or occasionally, a former smoker those who stopped smoking for at least six months, and a never smoker if the participant had never smoked [574]. Regarding alcohol intake, the participants were categorized as occasional drinkers if he/she had less than an alcoholic drink per day, daily drinker if he/she consumed, at least one drink per day, and a non-drinker if he/she did not consume any alcoholic beverages. Regular physical exercise was considered when the participant was engaged in some leisure-time physical activity performed on a repeated basis, spending at least 30 min a week. Four meteorological seasons were considered [575]. Participants evaluated in the June-November period (winter and fall) and December-May period (winter and spring) were classified, respectively, as "higher" and "lower" ultraviolet radiation exposure, according to a previous national study that showed 25(OH)D serum levels higher in summer and fall and lower in winter and spring [473].

Portuguese regions were classified as North, Center, Lisbon, Alentejo and Algarve according to the NUTS2 level statistical regions of the European Union.

Anthropometrics measures were taken by the same nurse that applied the questionnaire, namely weight, height, WC and HC. Body weight was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest centimetre in the standing position using a wall stadiometer. The WC was measured midway between the lower limit of the rib cage and the iliac crest and the HC was measured as the maximum circumference of the buttocks. BMI was calculated as the weight in kilogrammes divided by the square height in metres. The WHtR was calculated as the WC divided by the height, and the WHR, as the WC divided by the HC, all in centimeters. BAI [59] was calculated using the formula BAI = [(HC cm) / (Height m)$^{1.5}$)] – 18.

BP was measured on a single occasion using a standard mercury sphygmomanometer with the cuff on the upper right arm after a 10-min rest. Two BP readings were taken, and the mean of the two readings was calculated. If the difference between the two measures was larger than 5 mmHg for systolic or diastolic BP, a third measurement was acquired and the mean of the two closest values was registered.

A fasting venous blood sample was collected in each health care center and frozen samples were stored at – 80º C. All blood sampling analyses were performed centrally at the Department of Clinical Pathology, Centro Hospitalar São João, Porto, Portugal.

A chemiluminescent immunoassay using a Liaison automated analyzer (Diasorin Iberia, Madrid, Spain) was used to measure 25(OH)D. Biointact PTH and insulin were determined by an electro-chemiluminescent immunoassay using a Cobas e411 automated analyzer (Roche, Amadora, Lisboa, Portugal). High sensitivity C-reactive protein (hs-CRP) was measured using a particle-enhanced immunonephelometric

assay on a BN®II laser nephelometer. (Siemens Healthcare, Amadora, Lisboa, Portugal).

All other parameters [glucose, total cholesterol, triglycerides, HDL cholesterol, calcium, phosphorus, albumin, creatinine, TSH, free triiodothyronine (FT3), free thyroxine (FT4), TPOAb and TgAb] were measured using conventional methods with an Olympus AU5400® automated clinical chemistry analyser (Beckman-Coulter®,Oeiras, Lisboa, Portugal). All participants with triglycerides levels below 400 mg/dl had their LDL cholesterol level computed. This value was estimated by subtracting the HDL cholesterol value plus 20% of the triglycerides from the total cholesterol [576]. IR was estimated by the HOMA, from fasting glucose (mmol/L) and insulin (μUI/mL), as the product of the two divided by 22.5 [577].

Participants were classified according to the WHO criteria [578]: underweight, normal range, pre-obese and obese categories, which are defined as BMI < 18.5 kg/m^2, ≥18.5 to <25 kg/m^2, ≥ 25 to < 30 kg/m^2 and ≥ 30 kg/m^2, respectively.

Three operational definitions of MetS were used: the ATP III [4,5], IDF [10, 11], and JIS [1]. MetS was considered present by ATP III if at least three (any) of the following characteristics were present: fasting glucose ≥110 mg/dL; BP ≥ 130/85 mmHg; triglycerides ≥150 mg/dL; HDL cholesterol < 40 mg/dL in women and <30 mg/dL in men; WC > 88 cm in women and >102 cm in men. Participants who reported the use of antihypertensive or antidiabetic therapy were also considered as having the corresponding MetS feature by the ATP III classification. The considered IDF definition was WC ≥ 80 cm in women and ≥94 cm in men and the presence of at least two of the following characteristics: fasting glucose ≥100 mg/dL or previously

diagnosed type 2 diabetes; BP ≥ 130/85 mmHg or antihypertensive medication; triglycerides ≥150 mg/dL or current treatment for this lipid abnormality; HDL cholesterol < 40 mg/dL in women and <30 mg/dL in men or current treatment for this lipid abnormality. Finally, the JIS defined MetS as the presence of at least three (any) of the following characteristics: fasting glucose ≥100 mg/dL or antidiabetic treatment; BP ≥ 130/85 mmHg or antihypertensive medication; triglycerides ≥150 mg/dL or specific treatment for this lipid abnormality; HDL cholesterol < 40 mg/dL in women and <30 mg/dL in men or specific treatment for this lipid abnormality; WC ≥ 88 cm in women and ≥102 cm in men ("European" cut off points).

Elevated cardiometabolic risk was considered when two or more of the four criteria of MetS were present, excluding the WC component [46, 49].

VitD adequacy was classified according to the Institute of Medicine (IOM) recommended cut-off values for 25(OH)D levels [471]: deficiency below 12 ng/mL (30 nmol/L); inadequacy ≥ 12 and < 20 ng/mL (≥ 30 and < 50 nmol/L) and sufficiency ≥ 20 ng/mL (≥ 50 nmol/L).

Hyper- and hypoparathyroidism were defined as PTH levels above and below the standard laboratory reference range (10-65 pg/mL), respectively, and a "blunted PTH response" was defined as a PTH level within the reference range in the presence of 25(OH)D ≤ 12 ng/mL (30 nmol/L) [579].

Euthyroidism was defined by normal TSH (0.4 to 3.99 mU/L), FT4 (0.70 to 1.48 ng/dL) and FT3 (1.71 to 3.71 pg/mL) serum levels. Overt primary hypothyroidism was defined by a serum TSH level ≥ 4 mU/L and FT4 serum levels below the lower range. Subclinical hypothyroidism (SCH) was defined as a state of increased serum TSH

levels, with circulating thyroid hormone concentrations within the reference range. SCH was divided into two categories according to TSH levels: mildly increased TSH (4.0–10.0 mU/L) and severely increased TSH (>10 mU/L) [580]. Overt primary hyperthyroidism was defined as serum TSH <0.4 mU/L and serum FT4 and/or FT3 levels above the normal range. Subclinical hyperthyroidism (SHyper) was defined biochemically as serum TSH levels below the reference range, with normal thyroid hormone levels. According to its severity, SHyper was divided into two categories [581]: grade 1, which has low but detectable serum TSH levels (0.1–0.39 mU/l), and grade 2, which has undetectable serum TSH levels (<0.1 mIU/l).

Positivity for TPOAb and TgAb was set to values greater than or equal to 5.61 and 4.11 IU/mL, respectively.

IV. Results

Paper 1: Adiposity cut-off points for cardiovascular disease and diabetes risk in the Portuguese population: The PORMETS study.

Raposo L, Severo M, Santos AC.

PLoS ONE 2018;13(1): e0191641. doi: 10.1371/journal.pone.0191641.

PLOS ONE

RESEARCH ARTICLE

Adiposity cut-off points for cardiovascular disease and diabetes risk in the Portuguese population: The PORMETS study

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Citation: Raposo L, Severo M, Santos AC (2018) Adiposity cut-off points for cardiovascular disease and diabetes risk in the Portuguese population: The PORMETS study. PLoS ONE 13(1): e0191641. https://doi.org/10.1371/journal.pone.0191641

Editor: Pedro Tauler, Universitat de les Illes Balears, SPAIN

Received: December 23, 2016

Accepted: January 9, 2018

Published: January 29, 2018

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The authors received funding from "Bayer Health Care" to Luís Raposo and Fundação para a Ciência e a Tecnologia (IF/01060/2015) to Ana Cristina Santos. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors received funding from "Bayer Health Care". The funder had no role in study design, data collection and analysis, decision

Abstract

Objectives

The contribution of adiposity to cardiovascular and diabetes risk justifies the inclusion of an adiposity measure, usually waist circumference, in the definition of metabolic syndrome. However, waist circumference thresholds differ across populations. Our aim was to assess which adiposity measure performs the best in identifying the metabolic syndrome in a sample of Portuguese participants and to estimate cut-off values for these measures.

Methods

Data were obtained from a cross-sectional study (PORMETS study) conducted in Portugal between 2007 and 2009. A representative sample of non-institutionalized adults, comprising 3,956 participants, aged 18 years and older, was evaluated. A structured questionnaire was administered, collecting information on personal medical history, socio-demographics and behavioral characteristics. Anthropometrics, blood pressure and venous blood samples were also obtained. Metabolic syndrome was defined according to the Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology recommended criteria. Elevated cardiometabolic risk was considered when two or more of the four criteria of metabolic syndrome were present, excluding the waist circumference component. A receiver operating characteristic curve was used to estimate cut-off points.

Results

This study found that waist-to-height ratio, waist circumference and body adiposity index performed better than other adiposity measures, such as body mass index. The estimated cut-off points for waist-to-height ratio, waist circumference and body adiposity index in women and men were 0.564 / 89 cm / 27.4 and 0.571 / 93.5 cm / 25.5, respectively.

to publish, or preparation of the manuscript. This funding does not alter our adherence to PLOS ONE policies on sharing data and materials.

Conclusion

As waist circumference is currently used as the adiposity measure in the definition of metabolic syndrome and as no relevant differences were observed between this measure and waist-to-height ratio, it is likely that no modification to the metabolic syndrome definition needs to be proposed. Moreover, this study also confirmed the applicability of European cut-off points in the Portuguese population.

Introduction

The prevalence of obesity in Portugal [1] and worldwide [2] has risen to "pandemic" proportions. Obesity is independently related to type 2 diabetes [3], coronary heart disease, cerebrovascular disease and increased all-cause mortality as well as mortality after cardiovascular events [4, 5]. Despite the established contribution of overall and abdominal obesity to the cardiometabolic risk, visceral adipose tissue (VAT) may have a stronger impact than total body fat (TBF) on insulin resistance, beta cell dysfunction and atherosclerosis [6].

TBF can be estimated by weight (Wt), and other adiposity indicators that are often indexed to height (Ht) and include body mass index (BMI) and body adiposity index (BAI) [7]. Despite its good correlation with TBF, BMI is only moderately correlated with VAT [8].

To assess the distribution of adiposity, other indicators are available and calculated from anthropometric parameters [9], such as waist circumference (WC), hip circumference (HC) and several ratios, including waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR). When compared to BMI, WC and WHtR in both sexes and WHR in men presented stronger associations with VAT [10].

The superiority of measures of abdominal obesity (AO), namely WC and WHtR, over BMI for predicting cardiovascular disease (CVD) [11] and diabetes risk [3] has been increasingly documented, and a recent meta-analysis [12] even suggested that WHtR was modestly superior to WC for CVD risk assessment.

The contribution of obesity to CVD and diabetes risk justifies the inclusion of an adiposity measure, usually WC, in the most frequently used definitions of metabolic syndrome (MetS) [13]. MetS includes a group of interconnected clinical and metabolic factors that increases the risk of developing CVD in 5 to 10 years [14] and confers a 3- to 5-fold increased risk of type 2 diabetes [15]. These factors include dysglycemia, increased blood pressure, elevated triglyceride levels, low high-density lipoprotein cholesterol (HDL-C) levels, and AO.

The two most frequently used gender-specific thresholds for WC in the European population resulted from a proposal of the National Institutes of Health [16] based on a study in Caucasian populations [17]; this proposal established a BMI of $\geq$25 kg/m^2 or $\geq$30 kg/m^2 to correspond to WC cut-points of 80 or 88 cm for women and of 94 or 102 cm for men, respectively. However, an increasing number of published papers have documented a variation in the optimal cut-off values for WC in different populations [18, 19]. Therefore, the same methodology used to establish WC cut-off points may be applied to other adiposity measures.

The primary objective of the present study was to assess which adiposity measure performs the best in identifying MetS in a sample of Portuguese adults. The secondary objective was to estimate the cut-off values for these adiposity measures in the same sample.

Participants and methods

PORMETS is a cross-sectional study comprising a representative sample of adults registered at primary health care centers in mainland Portugal [20]. A cluster sample, representative of the Portuguese population, was obtained after stratification by selected districts and urban/rural areas. A total of 4,105 participants, aged 18 years and older, were evaluated, and the information was collected from February 2007 to July 2009. Ten women were excluded from the data analysis because they were pregnant at the time of the interview, resulting in 4,095 participants. After excluding participants with missing information on MetS, Ht, Wt, WC, and HC, the remaining 3,956 participants (2,287 women and 1,669 men) were included in the final data analysis (Fig 1).

PORMETS was approved by all Portuguese Regional Health Administrations, by the Ethics Committee of Centro Hospitalar São João (authorized in 27th February 2007) and by the Portuguese Data Protection Authority (authorization number: CNPD 1053/2007). Additionally, authorization was provided by the Clinical Director of each health care center. All participants provided written informed consent.

A structured questionnaire was administered by trained nurses collecting information on personal medical history and socio-demographic and behavioral characteristics, such as smoking, intake of alcoholic beverages and engagement in physical exercise.

Participants were considered current smokers if they smoked daily or occasionally, former smokers if they had stopped smoking for at least 6 months, and non-smokers if they had never smoked. Regarding alcohol intake, participants were categorized as occasional drinkers if they had less than one drink per day, daily drinkers if they consumed at least one drink per day, former drinkers if they had stopped drinking for at least 6 months and non-drinkers if they had never consumed any type of alcoholic beverage.

Adiposity measurements were collected, namely Wt, WC and HC. Body Wt was measured to 0.1 kg using a digital scale, and Ht to the nearest centimeter using a wall stadiometer with participants in the standing position. WC was measured midway between the bottom of the rib cage and the iliac crest, and HC was measured as the maximum circumference of the buttocks. BMI was calculated as Wt in kilograms divided by the square of Ht in meters.

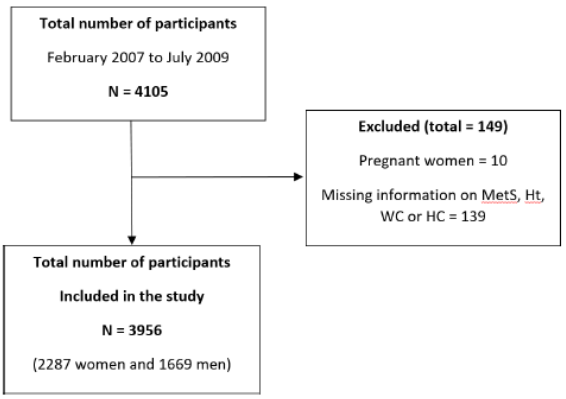

Fig 1. Flow chart of the PORMETS study. MetS, metabolic syndrome; Ht, height; Wt, weight; WC, waist circumference; HC, hip circumference.

https://doi.org/10.1371/journal.pone.0191641.g001

Participants were classified according to the World Health Organization criteria: overweight was defined as BMI $\geq$ 25 to $<$ 30 kg/m^2 and obesity as BMI $\geq$ 30 kg/m^2. The WHtR was calculated as the WC divided by the Ht, and the WHR, as the WC divided by the HC, all in centimeters. BAI [8] was calculated using the formula BAI = [(HC cm) / (Ht m)$^{1.5}$)]– 18.

Blood pressure was measured on a single occasion, using a standard mercury sphygmomanometer with the cuff on the right upper arm, after a 10-minute rest. Two blood pressure readings were obtained, and the mean of the two readings was registered. When the difference between the two measurements was larger than 5 mm Hg for systolic or diastolic blood pressure, a third measurement was taken and the mean of the two closest values was used.

A fasting venous blood sample was collected by trained nurses in each health care center. All blood sampling analyses were performed centrally at the Department of Clinical Pathology, Centro Hospitalar São João, Porto, Portugal. Glucose, total cholesterol, HDL-C and triglycerides were determined using automatic standardized routine enzymatic methods. High sensitivity C-reactive protein (hs-CRP) levels were determined by particle-enhanced immunonephelometry. Insulin was measured using a ^{125}I-labelled insulin radioimmunoassay method, and insulin resistance was estimated by the homeostatic model assessment (HOMA-IR) as the product of fasting glucose (mmol/L) and insulin (μUI/mL) divided by a constant 22.5 [21].

MetS was defined according to the Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology (JIS) recommended criteria [13]. MetS was considered present if at least three of the five following criteria were identified: fasting glucose $\geq$ 100 mg/dL (5.6 mmol/L) or antidiabetic treatment; blood pressure $\geq$130/85 mmHg or antihypertensive medication; triglycerides $\geq$150 mg/dL (1.7 mmol/L) or specific lipid-lowering therapy; HDL-C $<$50 mg/dL (1.29 mmol/L) in women and $<$40 mg/dL (1.03 mmol/L) in men or specific treatment for this lipid abnormality; and, using 2 cut-off points according to the European Cardiovascular Societies (*European*) or the International diabetes Federation (IDF) criteria (*Europid*), a WC greater than or equal to 102 or 94 cm in males and 88 or 80 cm in females, respectively.

Elevated cardiometabolic risk was considered present when two or more of the four JIS criteria were present, excluding the WC component [10, 22].

Statistical analysis

The association between the adiposity measures and adverse health outcomes (dichotomous variable) was estimated by point-biserial correlation.

Receiver operating characteristic (ROC) curves were created to compare the ability of several adiposity measures (Wt, BMI, HC, WC, WHtR, WHR and BAI) to identify elevated cardiometabolic risk and to estimate the cut-off points that better identified adverse health outcomes. The area under the ROC curve (AUC) was calculated for each adiposity measure, and the cut-off points were estimated using the point that maximized the sensitivity plus specificity. The application of this method was supported by its preferential use, as demonstrated in previous studies [18, 19]. The 95% confidence intervals (95% CI) for cut-off points were estimated using percentile bootstrap confidence interval calculations, based on 200 bootstrap replicate.

Sensitivity, specificity, positive and negative predictive values, accuracy, and positive and negative likelihood ratios (LR) were calculated for each estimated cut-off point.

Continuous variables were described as the means and standard deviations (SD) or as the median and corresponding 25th and 75th percentiles for non-normally distributed variables. Counts and proportions were reported for categorical variables. Proportions were compared

using a chi-square test or Fisher's exact test, as appropriate. T-tests for two independent samples or Mann-Whitney test were also used to compare continuous variables. A p value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS® version 21 (IBM, Armonk, New York, US).

Results

This study comprised 3,956 individuals, of whom 2,287 were women (57.8%). The mean (±SD) age in this sample was 53.2 (±16.3) years; women were 52.6 (±16.3) years old on average, and men were 54.1 (±16.4) years old. In general, women had lower systolic and diastolic blood pressure ($p<0.001$), fasting glucose ($p<0.001$) and triglycerides ($p<0.001$) but higher HDL-C ($p<0.001$), insulin ($p = 0.002$) and hs-CRP ($p<0.001$) levels than men (Table 1).

For all anthropometric measures there were significant differences between genders (Table 2). Women showed higher values than men for BMI ($p = 0.003$), HC ($p<0.001$), WHtR ($p = 0.003$) and BAI ($p<0.001$), but lower values of WC ($p<0.001$) and WHR ($p<0.001$).

The prevalence of elevated cardiometabolic risk was 52.8% (48.3% in women and 58.9% in men) and the prevalence of its components was 62.0%, 55.2%, 24.8% and 23.5% for blood pressure, triglycerides, fasting glucose and HDL-C, respectively. Overall, men presented a higher prevalence of all outcome components ($p<0.001$) with the exception of HDL-C.

Table 3 shows the correlations (95% CI), the cut-off points (95% CI), the AUC (95% CI), the performance parameters and the MetS and adiposity component prevalence for all the

Table 1. Sample demographic, behavioral and analytical characteristics according to gender.

	Women	Men	p-value
Overall [N (%)]	2287 (57.8)	1669 (42.4)	
Age (years)–mean (SD)	52.6 (16.3)	54.1 (16.4)	0.004
Education (years)–mean (SD)	6.7 (4.7)	6.8 (4.3)	0.562
Regular physical exercise [N (%)]	561 (24.5)	495 (29.7)	<0.001
Smoking			
Never smoker [N (%)]	1934 (85.5)	822 (50.3)	
Former smoker [N (%)]	116 (5.1)	452 (27.6)	
Current smoker [N (%)]	212 (9.4)	361 (22.1)	<0.001
Alcohol intake			
Non-drinker [N (%)]	1501 (66.1)	284 (17.1)	
Former drinker [N (%)]	55 (2.4)	81 (4.9)	
Occasional drinker [N (%)	517 (22.8)	515 (31.0)	
Daily drinker [N (%)]	199 (8.8)	779 (47.0)	<0.001
Systolic BP (mmHg)—Mean (SD)	129.0 (22.2)	135.9 (21.8)	<0.001
Diastolic BP (mmHg)—Mean (SD)	77.2 (12.1)	79.8 (12.1)	<0.001
Total cholesterol (mg/dL)—Mean (SD)	209.7 (39.7)	207.5 (44.8)	0.114
Triglycerides (mg/dL)—Mean (SD)	115.3 (58.6)	134.8 (85.0)	<0.001
HDL-C (mg/dL)—Mean (SD)	50.9 (11.8)	43.8 (12.3)	<0.001
Fasting glucose (mg/dL)—Mean (SD)	88.72 (25.50)	96.49 (30.60)	<0.001
Insulin (μIU/mL)–median (P25, P75)	7.8 (5.3, 11.5)	7.4 (4.7, 11.6)	0.002
HOMA-IR–median (P25, P75)	1.64 (1.07, 2.53)	1.66 (1.01, 2.74)	0.694
hs-CRP (mg/L)–median (P25, P75)	0.19 (0.08, 0.44)	0.13 (0.07, 0.27)	<0.001

SD, standard deviation; BP, blood pressure; HOMA-IR, homeostatic model assessment-insulin resistance; hs-CRP, high sensitivity C-reactive protein.

https://doi.org/10.1371/journal.pone.0191641.t001

Table 2. Adiposity measures and frequency of metabolic syndrome and its individual components according to gender.

	Women	Men	p-value
Mean (SD)			
Weight (kg)	68.0 (12.8)	78.1 (13.0)	<0.001
Ht (cm)	157.2 (6.7)	169.7 (7.2)	<0.001
WC (cm)	91.0 (12.5)	97.0 (11.4)	<0.001
HC (cm)	104.5 (10.5)	102.3 (8.3)	<0.001
WHtR	0.580 (0.085)	0.572 (0.070)	0.003
WHR	0.870 (0.078)	0.947 (0.072)	<0.001
BAI	28.3 (7.2)	26.0 (5.7)	<0.001
BMI (kg/m^2)	27.6 (5.1)	27.1 (4.0)	0.003
N (%)			
Overweight (≥ 25 BMI < 30)	864 (38.4)	773 (47.0)	<0.001
Obesity (BMI ≥ 30)	619 (27.5)	373 (22.7)	0.001
Elevated cardiometabolic risk	1096 (48.3)	977 (58.9)	<0.001
Blood pressure MetS component	1305 (57.1)	1145 (68.6)	<0.001
HDL-C MetS component	1345 (58.9)	838 (50.2)	<0.001
Triglycerides MetS component	468 (20.5)	513 (30.8)	<0.001
Fasting glucose MetS component	417 (18.3)	507 (30.5)	<0.001

SD, standard deviation; Ht, height; WC, waist circumference; HC, hip circumference; WHtR, waist-to-height ratio; WHR, waist-to-hip ratio; BAI, body adiposity index; BMI, body mass index; MetS, metabolic syndrome (according to the JIS definition).

https://doi.org/10.1371/journal.pone.0191641.t002

evaluated adiposity measures. The ability of the adiposity measures to identify elevated cardiometabolic risk, based on the AUC are presented in Fig 2.

WC and WHR showed a good sensitivity for both sexes (74.6% / 78.1% in women and 76.8% / 77.2% in men, respectively); additionally, WHtR and BAI performed well in women (75.9% and 74.5%, respectively). The specificity was low for all cut-off points, especially in women. The WHtR and BAI presented higher and lower positive and negative LR, respectively.

Overall, WC, WHtR, and BAI cut-off points showed a good performance in both sexes, in terms of sensitivity, specificity, predictive value, accuracy and LR.

Using the JIS criteria, and according to the cut-off points tested for all adiposity measures, the prevalence of MetS varied between 37.7% and 41.3% in women and between 44.0% and 49.6% in men. Considering the estimated WC cut-off points, MetS prevalence was 39.6% in women and 49.2% in men. The WC cut-off points determined for this Portuguese sample were closer to the *European* criteria in women and to the *Europid* criteria in men [13].

The prevalence of AO using the estimated WC cut-off points (≥ 89.0 cm in women and ≥ 93.5 cm in men) was 56.9% in women and 62.7% in men.

Discussion

In this study, the correlations of WHtR, WC and BAI with elevated cardiometabolic risk were higher than those of other adiposity measures, namely, BMI. These results are supported by other published evidence, which showed that WC and WHtR performed better in detecting CVD and diabetes risk than BMI [3, 11, 12, 22]. In addition, the results are partially explained by the stronger association of those anthropometric measures with VAT [9, 10]. However, BAI performed quite well in our sample, which is not supported by other studies [10, 23, 24].

Table 3. Adiposity measures, elevated cardiometabolic risk and cut-off points performance.

	Elevated CMR correlations (95% CI) [a]	AUC (95% CI)	Cut-off points (95% CI)	Sn (%)	Sp (%)	PPV (%)	NPV (%)	Accuracy (%)	LR +	LR -	Adipose Comp. (%)	MetS (%)
Weight(kg) W	0.276 (0.237, 0.313)	0.664 (0.642, 0.686)	65.0 (63.1, 66.2)	69.4	56.5	59.9	66.4	62.8	1.595	0.542	56.0	38.1
M	0.239 (0.196, 0.285)	0.648 (0.621, 0.674)	77.0 (73.2, 79.4)	60.4	62.2	69.6	52.3	61.1	1.598	0.637	51.2	44.0
BMI(kg/m^2) W	0.352 (0.317, 0.387)	0.714 (0.693, 0.735)	26.5 (25.3, 27.2)	71.2	61.3	63.2	69.5	66.1	1.840	0.470	54.4	38.9
M	0.340 (0.300, 0.381)	0.709 (0.684, 0.734)	27.0 (25.8, 27.1)	63.1	69.2	74.6	56.7	65.6	2.049	0.533	49.9	44.7
WC (cm) W	0.400 (0.368, 0.435)	0.733 (0.713, 0.754)	89 (85.8, 91.6)	74.6	59.8	63.4	71.6	66.9	1.856	0.425	56.9 [b]	39.6 [c]
M	0.376 (0.336, 0.418)	0.731 (0.706, 0.756)	93.5 (91.8, 99.8)	76.8	57.5	72.1	63.3	68.8	1.807	0.403	62.7 [d]	49.2 [e]
HC (cm) W	0.287 (0.249, 0.324)	0.665 (0.643, 0.687)	103.0 (100.0, 108.1)	66.8	56.6	59.0	64.6	61.5	1.539	0.587	54.8	37.7
M	0.229 (0.187, 0.275)	0.636 (0.608, 0.663)	99.0 (97.3, 104.2)	71.4	48.4	66.5	54.2	62.0	1.384	0.591	63.4	48.2
WHtR W	0.423 (0.389, 0.458)	0.747 (0.727, 0.767)	0.564 (0.549, 0.592)	75.9	61.6	64.9	73.3	68.5	1.977	0.391	56.5	39.9
M	0.406 (0.367, 0.444)	0.746 (0.722, 0.770)	0.571 (0.551, 0.584)	66.3	71.6	77.0	59.7	68.5	2.335	0.471	50.9	45.9
WHR W	0.303 (0.266, 0.338)	0.678 (0.656, 0.699)	0.844 (0.834, 0.883)	78.1	48.0	58.4	70.1	62.5	1.502	0.456	64.6	41.3
M	0.342 (0.299, 0.382)	0.709 (0.684, 0.735)	0.930 (0.922, 0.933)	77.2	58.4	72.6	64.1	69.4	1.856	0.390	62.6	49.6
BAI W	0.421 (0.387, 0.456)	0.746 (0.726, 0.766)	27.4 (26.2, 28.5)	74.5	64.3	66.1	72.9	69.2	2.087	0.397	54.5	39.5
M	0.402 (0.365, 0439)	0.742 (0.717, 0.766)	25.5 (24.9, 26.5)	70.0	68.5	76.1	61.4	69.4	2.222	0.438	54.3	47.4

W, women; M, men; CMR, cardiometabolic risk; AUC, area under the ROC curve; Sn, sensitivity; Sp, Specificity; PPV, positive predictive value; NPV, negative predictive value; LR, likelihood ratio; Comp., component of the metabolic syndrome; MetS, metabolic syndrome (according to the JIS definition); WC, waist circumference; HC, hip circumference; WHtR, waist-to-height ratio; WHR, waist-to-hip ratio; BAI, body adiposity index; BMI, body mass index.

[a] $p<0.001$ for all the correlation coefficients

[b] According to the *European* and *Europid* cut-off points for WC the prevalence was 60.2% and 81.0% in women

[c] According to the *European* and *Europid* cut-off points for WC the prevalence was 40.6% and 45.9% in women

[d] According to the *European* and *Europid* cut-off points for WC the prevalence was 32.7% and 62.1% in men

[e] According to the *European* and *Europid* cut-off points for WC the prevalence was 38.8% and 49.0% in men.

https://doi.org/10.1371/journal.pone.0191641.t003

As expected, differences between genders were observed for the correlations between elevated cardiometabolic risk and the evaluated adiposity measures, as well as for the estimated cut-off points. These differences may be explained by the previously described gender variations in body fat distribution [25, 26]. Compared to women, men have greater total VAT volume and are more likely to accumulate adipose tissue in their upper body [27], whereas women usually accumulate adipose tissue in the lower body. This fact may explain why men show higher VAT values than women for the same WC value [28].

The WHR cut-off points showed a high sensitivity and low specificity in both sexes. However, WHR performed better in men than in women when considering the positive predictive value, accuracy and LR; these observations are supported by previously published studies [29]. The gender differences in body fat distribution could, at least in part, explain the differences in CVD and diabetes risk observed between men and women [30].

According to our data, WHtR was the adiposity measure that best performed in the evaluation of the adiposity component for MetS. As WC is currently used as the adiposity component in the definition of MetS and as no significant differences were observed between this and other measures in our study, it is likely that no modification to the MetS definition need to be proposed. Regarding the good performance of WHtR, this measure may be useful in benchmarking studies of different populations as it allows for differences in Ht [31, 32].

The estimated cut-off points for WC obtained in this study were 89.0 cm in women and 93.5 cm in men. These cut-off points are lower in men than the ones proposed by the National Institute of Health [16]. Therefore, these results suggest that, in the Portuguese population, the

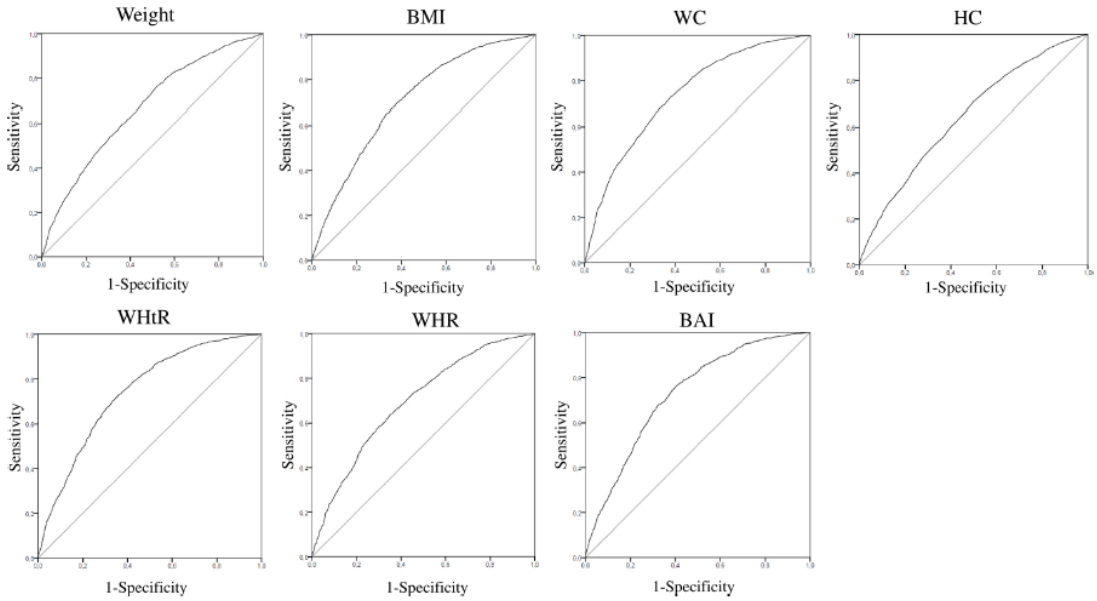

Fig 2. ROC curves of several adiposity measures in terms of the elevated cardiometabolic risk outcome. WHR: waist-to-hip ratio; BMI: body mass index; WC: waist circumference; BAI: body adiposity index; WHtR: waist-to-height ratio.

https://doi.org/10.1371/journal.pone.0191641.g002

European cut-off points appear to be more appropriate to prevent over-diagnosis in women. According to the *Europid* cut-offs, the majority of women (81%) met the criteria for the WC component. However, men with a WC of 94.0 cm or more should be carefully assessed.

Recently published results from Spanish studies [33, 34] showed similar cut-off points for WC (88.5 to 89.5 cm in women and 94.5 cm in men), with close WC mean values in men and discretely higher values in women. This similarity with our results may reflect a genetic and epigenetic proximity between the populations. A review, including sixty-one research papers [18], found optimal cut-off values ranging from 65.5 to 101.2 cm for women and 72.5 to 103.0 cm for men; the cut-off points of the European and United States studies were not very different from ours (83 to 88 cm in women and 93 to 96 cm in men). Another review, including studies investigating ethnic-specific WC cut-off points among Aboriginal, Asian, African (SubSaharan), African-American, Hispanic, Middle Eastern, Pacific Islander and South American populations [19], found lower cut-off point in the populations of Asian origin; the evidence was less robust for other ethnic groups.

When comparing the prevalences of MetS, according to the JIS definition, the estimated and proposed (*European* and *Europid*) WC cut-off points showed a closer prevalence between the figure estimated using the *European* cut-off points in women and the *Europid* cut-off points in men.

Furthermore, we found a higher prevalence of MetS in the Portuguese sample than in other South European countries [35–38] and the USA [39]. In addition, the estimated prevalence of AO (based on WC) and overweight was slightly higher in this sample than in previous national studies [40].

Overall, our study was based on cross-sectional data. Prospective studies should be conducted to determine whether the estimated WC cut-off values are more appropriate than the *European* and *Europid* values and, moreover, to estimate the risk of CVD and diabetes in the Portuguese population.

Conclusions

According to our data, WC, WHtR and BAI are the adiposity measures that provided the best evaluation of the adiposity component for MetS in the Portuguese population.

As WC is currently used as the adiposity measure in the definition of MetS and as no relevant differences were observed between this measure and WHtR, it is likely that no modification to the MetS definition need to be proposed.

The new WC cut-off points proposed for the Portuguese population (89.0 cm in females and 93.5 cm in males) are very similar to the *European* cut-off points in women and the *Europid* values in men.

Use of the *European* cut-off points may be more appropriate in order to prevent over-diagnosis in women.

Supporting information

S1 Supporting information. Data set.
(SAV)

Acknowledgments

The authors thank all the participants in the PORMETS cohort, the logistic staff and the scientists for their contribution to the study.

Author Contributions

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Funding acquisition: Luís Raposo, Ana Cristina Santos.

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Paper 2: The prevalence of the metabolic syndrome in Portugal: the PORMETS study.

Raposo L, Severo M, Barros H, Santos AC.

BMC Public Health 2017;17:555. doi: 10.1186/s12889-017-4471-9.

Raposo et al. BMC Public Health (2017) 17:555
DOI 10.1186/s12889-017-4471-9

BMC Public Health

RESEARCH ARTICLE Open Access

The prevalence of the metabolic syndrome in Portugal: the PORMETS study

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Abstract

Background: The PORMETS study was designed to estimate the prevalence of metabolic syndrome and its determinants in the overall and administrative regions of the Portuguese mainland.

Methods: A cross-sectional study of a representative sample of non-institutionalized Portuguese adults selected from primary health care centres lists including 1695 men and 2309 women was conducted from February 2007 to July 2009. A structured questionnaire was administered, collecting information on personal medical history and socio-demographic and behavioural characteristics. Anthropometrics, blood pressure, and venous blood samples were obtained. Metabolic syndrome was defined according to three operational definitions. The prevalence ratios and their respective 95% confidence intervals were calculated using binomial generalized linear regression, with the log link function.

Results: The prevalence rates of metabolic syndrome in this sample of Portuguese adults were 36.5%, 49.6%, and 43.1%, using the Adult Treatment Panel III, International Diabetes Federation and Joint Interim Statement definitions, respectively. The most prevalent feature of metabolic syndrome in this sample was high blood pressure (64.3%) and the lowest was high fasting glucose (24.9%). After adjustment for age and gender, significant differences were observed for the 18 districts of the Portugal mainland. Additionally, metabolic syndrome was significantly more frequent in non-urban areas than in urban ones ($p = 0.001$). The prevalence of metabolic syndrome was significantly higher in women ($p^{*}0.001$) and older participants ($p^{*}0.001$), as well as in those who reported being housewives ($p = 0.010$), retired ($p = 0.046$) or unemployed ($p = 0.024$).

Conclusions: This study showed that metabolic syndrome is highly prevalent in the Portuguese adult population. Regional differences in the prevalence of this syndrome were observed, and this condition was more common in non-urban areas and less favoured socio-economic categories.

Keywords: Metabolic syndrome, Prevalence, Portugal, Rural, Regional, Socio-economic

Background

Metabolic syndrome (MetS) is a cluster of interrelated risk factors for cardiovascular disease (CVD) [1] and diabetes [2]. These factors include dysglycaemia, elevated blood pressure, elevated triglyceride levels, low high-density lipoprotein cholesterol (HDL-C) levels, and central obesity. Different organizations have proposed several diagnostic criteria [3–6]. However, finally, a consensus was established by the International Diabetes Federation (IDF), National Heart, Lung, and Blood Institute, American Heart Association and other international societies [7].

MetS is a common condition and its worldwide prevalence is rising [8], which can be related to the increase in obesity rates and sedentary lifestyles, as well as regional prevalence of hypertension and diabetes. The prevalence of overweight and obesity in Portugal is high [9] and is increasing [10]. Overall, individuals with normal weight (Wt) represent less than 50% of the adult population [10]. Recent studies have also shown a high prevalence of hypertension and type 2 diabetes in the Portuguese population (42.2% and 11.7%, respectively) [11, 12].

Previous national studies have shown the prevalence of MetS, ranging from 42% to 66%, according to the IDF

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criteria [13–15]. However, none of them approached district and urban versus non-urban differences.

The Portuguese Metabolic Syndrome (PORMETS) study, was designed to assess the prevalence of MetS and its determinants in the overall and administrative regions of the Portuguese mainland.

Methods

PORMETS is a national cross-sectional study. A sample of adults registered at primary health care centres throughout mainland Portugal was selected. In each of the eighteen Portuguese mainland administrative regions (districts), two health care centres were included, one in the district's capital and the other representative of a non-urban area. Apart from the district of Setubal that only included one centre, all the others included two, for a total of 35 health care centres. At each centre, the participants were randomly selected from the general practitioner's patient lists, and 120 participants were evaluated, with an inclusion criterion of being aged 18 or older. A total of 4105 participants was evaluated, and information was collected from February 2007 to July 2009. Ten participants were excluded from the data analysis because they were pregnant at the time of the interview, resulting in 4095 remaining participants. After excluding participants who had missing information on the MetS features, the final analysis included 4004 participants, 2309 women and 1695 men.

All of the Portuguese Regional Health Administrations, the Ethics Committee of the São João Hospital E.P.E. and the Portuguese Data Protection Authority approved PORMETS. The Clinical Director of each health care centre also provided authorization and all participants provided written informed consent.

Anthropometrics measures were recorded, namely Wt, height, and waist and hip circumferences. Body Wt was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest centimetre in the standing position using a wall stadiometer. Body mass index (BMI) was calculated as the Wt in kilogrammes divided by the square height in metres. The waist circumference (WC) was measured midway between the lower limit of the rib cage and the iliac crest and the hip circumference (HC) was measured as the maximum circumference of the buttocks. Blood pressure was measured on a single occasion using a standard mercury sphygmomanometer with the cuff on the upper right arm after a 10-min rest. Two blood pressure readings were taken, and the mean of the two readings was calculated. If the difference between the two measures was larger than 5 mmHg for systolic or diastolic blood pressure, a third measurement was acquired and the mean of the two closest values was registered.

Fasting venous blood glucose, total cholesterol and triglycerides levels were determined using automatic standard routine enzymatic methods. HDL-C was determined after the precipitation of apolipoprotein B-containing lipoproteins. All participants with triglycerides levels below 400 mg/dl had their LDL cholesterol level computed. This value was estimated by subtracting the HDL- C value plus 20% of the triglycerides from the total cholesterol. High sensitivity C-reactive protein (hs-CRP) levels were determined using particle-enhanced immunonephelometry. Insulin was measured using the ^{125}I–labelled insulin radioimmunoassay method, and insulin resistance was estimated according to the homeostatic model assessment (HOMA), as the product of fasting glucose (mmol/L) and insulin (μUI/mL) divided by a constant 22.5.

A trained nurse administered a structured questionnaire with only closed ended questions; information was collected on personal medical history and socio-demographic and behavioural characteristics. A participant was considered a current smoker if he/she smoked daily or occasionally, a former smoker was considered a participant who had stopped smoking for at least six months, and a never smoker was considered a participant who had never smoked [16]. Regarding alcohol intake, the participants were categorized as occasional drinkers if he/she had less than a drink per day, daily drinker if he/she consumed, at least one drink per day, and a non-drinker if he/she did not consume any alcoholic beverages. Regular physical exercise was considered when the participant was engaged in some leisure-time physical activity performed on a repeated basis, spending at least 30 min a week.

Portuguese regions were classified as North, Center, Lisbon, Alentejo and Algarve according to the NUTS2 level statistical regions of the European Union.

Three operational definitions of MetS were used: the Adult Treatment Panel III (ATP III) [4], IDF [5], and HARM2009 [7]. MetS was considered present by ATP III if at least three (any) of the following characteristics were present: fasting glucose ≥110 mg/dL; blood pressure ≥ 130/85 mmHg; triglycerides ≥150 mg/dL; HDL-C < 40 mg/dL in women and <30 mg/dL in men; WC > 88 cm in women and >102 cm in men. Participants who reported the use of antihypertensive or antidiabetic therapy were also considered as having the corresponding MetS feature by the ATP III classification. The considered IDF definition was WC ≥ 80 cm in women and ≥94 cm in men and the presence of at least two of the following characteristics: fasting glucose ≥100 mg/dL or previously diagnosed type 2 diabetes; blood pressure ≥ 130/85 mmHg or antihypertensive medication; triglycerides ≥150 mg/dL or current treatment for this lipid abnormality; HDL-C < 40 mg/dL in women and <30 mg/dL in men or current treatment for this lipid abnormality. Finally, the HARM2009 defined

MetS as the presence of at least three (any) of the following characteristics: fasting glucose ≥100 mg/dL or antidiabetic treatment; blood pressure ≥ 130/85 mmHg or antihypertensive medication; triglycerides ≥150 mg/dL or specific treatment for this lipid abnormality; HDL-C < 40 mg/dL in women and <30 mg/dL in men or specific treatment for this lipid abnormality; WC ≥ 88 cm in women and ≥102 cm in men ("European" cut off points).

Statistical analysis

The data are described as the mean values and standard deviation (SD) or as median values and corresponding 25th and 75th percentiles for non-normally distributed variables. Counts and proportions were reported for categorical variables. Proportions were compared using the chi-square test or *Fisher's* exact test, whenever appropriate. Student's t test or Mann-Whitney *U*-test was used to compare continuous variables.

The prevalence of MetS and its individual components was age and sex adjusted, using binomial generalized linear regression, with the log link function.

The prevalence and prevalence ratio (PR) of MetS and their respective 95% confidence intervals (95%CI) were estimated for districts and NUT II by binomial generalized linear regression, with the log link function. Each district and NUT II region was compared with the overall effect, obtained as the pooled geometric mean prevalence of all districts, and the PR and respective 95%CI were calculated using the deviation contrast method [17].

To estimate the magnitude of association between MetS and demographic, socio-economic analytical and lifestyle characteristics, the PR and 95%CI were also computed using binomial generalized linear regression, with the log link function. The *p*-values were obtained using the Wald test from the respective generalized linear regressions.

Statistical analysis was performed using SPSS version 21 * and R version 8.0 software (R Foundation, Vienna, Austria).

Results

This study included 4004 individuals, 2309 women and 1695 men with a mean age of 53.2 (SD = 16.3) years. The mean age was 54.1 (SD = 16.3) years in men and 52.6 (SD = 16.3) years in women ($p = 0.004$). The mean (SD) levels of -glucose, triglycerides and HDL-C in women and men were, respectively, 88.7 (25.4) / 115.1 (58.5) / 50.9 (11.8) and 96.5 (30.5) / 135.1 (85.0) / 43.8 (12.3) mg/dL. The mean (SD) values of WC were 90.9 (12.5) cm in women and 97.0 (11.4) cm in men. Systolic and diastolic blood pressure values (mean and SD) were, respectively, 129.1 (22.2) / 77.2 (12.1) mmHg in women and 136.0 (21.8) / 79.8 (12.1) mmHg in men. Differences between sexes were significant ($p < 0.001$) for all.

The prevalence rates of MetS in this sample of Portuguese adults adjusted for gender and age were 36.5%, 49.6% and 43.1% (crude prevalence rates of 32.7%, 45.9% and 40.0%) using the ATPIII, IDF and HARM2009 definitions respectively (Table 1). The most prevalent feature of MetS in this sample was "high blood pressure" (64.3%) and the lowest was "high fasting glucose" (24.9%). Most participants with MetS had 3 features (23.3%). A minority had 5 features (4.9%). Significant differences in gender were observed in the prevalence rates of MetS ($p < 0.001$), MetS features ($p < 0.001$) and number of MetS features ($p = 0.044$). Women showed a significantly higher prevalence of "high WC" and "low HDL-C" components; all the other features were more prevalent in men. The prevalence of the number of MetS features also varied according to sex. The presence of one or less components was higher in men, and the presence of two to four components was higher in women. Regarding the geographical distribution of MetS and after adjustment for age and gender, significant differences were observed for the 18 districts of the Portugal mainland (Table 2); "Vila Real", and "Leiria" districts had a higher prevalence of MetS; however, "Bragança" and "Beja" districts presented a lower prevalence. However, no differences were observed when we compared the syndrome prevalence according to NUTS2 statistical regions, which establish a north-south division of the country (Table 3).

In Table 4, results from the comparison between participants with and without MetS are presented according to demographic, behavioural, anthropometric and analytical characteristics. As expected, participants with MetS had significantly higher mean values of Wt, BMI and WC. Regarding analytical characteristics, participants with MetS had significantly higher mean levels of glucose and triglycerides ($p < 0.001$). The insulin level and HOMA were also significantly higher in participants with MetS ($p < 0.001$). In addition, individuals with MetS syndrome significantly reported a higher prevalence of previously diagnosed type 2 diabetes ($p < 0.001$), myocardial infarction ($p < 0.001$) and stroke ($p = 0.001$). MetS was significantly more frequent in women and older subjects ($p < 0.001$). In addition, the syndrome was more common in housewives ($p = 0.010$), retired ($p = 0.046$) or unemployed ($p = 0.024$) participants. However, MetS was less frequent in smokers ($p = 0.001$) and in those that reported regular physical exercise ($p < 0.001$).

When we compared the prevalence of the syndrome, according to the classification of the health care centre location in an urban/non-urban area, we found that MetS was significantly ($p = 0.001$) more prevalent in non-urban subjects after adjustment for sex and age (PR:1.13;95%CI of 1.05 to 1.205). Non-urban residents

Table 1 Metabolic syndrome age-adjusted prevalence and its individual features according to different proposed definitions

	n	Total [% (95%CI)]	Women [% (95%CI)]	Men [% (95%CI)]	*p*
MetS definition					
ATP III	3986	36.5 (34.3–38.6)	38.8 (36.2–41.4)	33.5 (30.8–36.2)	<0.001
IDF	3986	49.6 (47.5–51.7)	52.0 (49.5–54.5)	46.5 (43.9–49.1)	<0.001
HARM2009	3987	43.1 (41.0–45.3)	45.7 (43.2–48.3)	39.8 (37.2–42.4)	<0.001
MetS features [a]					
Waist circumference	3977	51.0 (48.9–53.1)	66.2 (63.6–68.7)	35.7 (33.2–38.2)	<0.001
Glucose	3965	24.9 (23.0–26.8)	20.8 (18.8–22.8)	32.0 (29.2–34.8)	<0.001
Triglycerides	3980	29.4 (27.3–31.5)	24.7 (22.5–27.0)	37.2 (34.1–40.4)	<0.001
HDL cholesterol	3984	56.5 (54.4–58.6)	61.2 (58.6–63.7)	50.6 (47.9–53.3)	<0.001
Blood pressure	3984	64.3 (60.8–67.8)	60.6 (56.6–64.5)	69.7 (64.9–74.5)	<0.001
Number of MetS features [a]					
0	530	8.9	8.4	9.7	
1	846	20.4	19.3	21.8	
2	998	26.2	25.9	26.5	
3	874	23.3	24.9	21.2	
4	518	16.3	16.6	15.7	
5	182	4.9	4.9	5.0	
Mean (95%CI)		2.29 (2.23–2.35)	2.32 (2.26–2.39)	2.24 (2.17–2.31)	0.044

MetS: metabolic syndrome.
The PORMETS study was conducted in mainland Portugal from February 2007 to July 2009.
[a]Metabolic syndrome features defined according to HARM2009.

were discretely older (mean age of 53.5 versus 52.9 years; $p = 0.229$), presenting a slightly higher proportion of men (44.0 versus 40.8%; $p = 0.040$) and had a lower education level (mean of 6.0 versus 7.5 schooling years; $p < 0.001$). After additional adjustment for the education level, non-urban residents maintained a higher PR for MetS (1.17; 95%CI of 1.06 to 1.29).

Discussion

The crude prevalence of MetS in our population-based survey varied according to the definition used. The HARM2009 definition, supported by several major organizations [7], is the most consensual. The ATP III and IDF definitions have been widely used and may be useful for comparisons of the prevalence of MetS between studies. The prevalence was lowest according to the ATP III definition (32.7%), followed by the HARM2009 definition (40%), and was highest when using the IDF definition (45.9%). These differences were confirmed by previous studies [13]. The cut-off points of the WC component in the IDF setting were lower, and the cut-off points of glycaemia in the ATP III definition were higher than those using the HARM2009 definition.

The high prevalence of MetS is supported by previous Portuguese studies [13–15]. A study in the city of Porto, conducted during 1999–2003, and including a younger population (mean age of 52.5 years) recruited with a different methodology, estimated lower MetS crude prevalence rates (24.0%, 41.9% and 27.6% according to the ATP III, IDF and HARM2009 definitions, respectively) [13]. Another national study [15], carried out between 2006 and 2007, with different selection criteria and including an older population (mean age of 58.1 years), observed crude MetS prevalence rates of 28.4%, 65.5% and 69.4%, respectively, discretely higher than those in the present study. The PREVADIAB study conducted from 2008 to 2009 showed a crude prevalence of 41.5% by IDF criteria [14]. Nevertheless, the national prevalence of MetS is higher than in some European countries and the USA [8].

The high prevalence of MetS in the Portuguese population may partly be explained by the decreasing adherence of the Portuguese population to the Mediterranean diet [18] and high prevalence of sedentary lifestyles, namely in older adults [19], hypertension [11], obesity [9] and type 2 diabetes [12].

Hypertension is highly prevalent among Portuguese adults [11] but, as observed in other countries [20], a decreasing trend in the last decade was observed [21].

The prevalence of obesity in Portugal [9] and worldwide [22] has risen to epidemic/pandemic proportions. A systematic review on the prevalence of overweight and obesity in Portugal (1995–2005) showed an increase in the overweight prevalence by 3.2% and 3.5%

Table 2 Metabolic syndrome prevalence (as defined by HARM2009) by Portuguese district

District	Prevalence (95%CI)	Prevalence ratio (95%CI)	Prevalence (95%CI)	Prevalence ratio (95%CI)
	Crude		Adjusted for age and sex	
Viana do Castelo	42.3 (35.8–48.8)	1.07 (0.91–1.23)	45.9 (39.6–52.1)	1.06 (0.92–1.20)
Braga	28.5 (22.7–34.2)	0.72 (0.59–0.86)	39.2 (32.1–46.2)	0.91 (0.75–1.07)
Vila Real	50.6 (44.2–57.0)	1.28 (1.12–1.44	55.1 (49.3–60.9)	1.27 (1.15–1.39)
Bragança	31.7 (25.8–37.6)	0.80 (0.66–0.95)	35.9 (29.7–42.1)	0.83 (0.70–0.97)
Porto	47.6 (41.1–54.1)	1.20 (1.04–1.37)	47.3 (41.4–53.2)	1.10 (0.97–1.22)
Aveiro	39.5 (33.2–45.8)	1.00 (0.85–1.16)	42.4 (36.2–48.7)	0.98 (0.85–1.11)
Viseu	29.4 (23.5–35.2)	0.74 (0.61–0.89)	38.4 (31.7–45.1)	0.89 (0.74–1.04)
Guarda	43.6 (37.2–49.9)	1.10 (0.95–1.26)	45.1 (39.1–51.1)	1.04 (0.91–1.17)
Coimbra	34.7 (28.7–40.7)	0.88 (0.74–1.03)	39.8 (33.4–46.1)	0.92 (0.78–1.06)
Leiria	48.0 (41.1–54.9)	1.21 (1.04–1.39)	50.7 (44.4–57.0)	1.17 (1.03.1.31)
Castelo Branco	44.0 (37.7–50.3)	1.11 (0.96–1.27)	44.7 (38.8–50.6)	1.04 (0.91–1.16)
Santarém	38.1 (31.9–44.3)	0.96 (0.82–1.12)	43.8 (37.5–50.1)	1.01 (0.88–1.15)
Lisbon	46.0 (39.7–52.3)	1.16 (1.01–1.32)	40.2 (34.7–45.7)	0.93 (0.81–1.05)
Portalegre	43.5 (36.0–50.9)	1.10 (0.92–1.28)	49.4 (42.5–56.2)	1.14 (0.97–1.30)
Évora	41.4 (35.2–47.7)	1.05 (0.90–1.20)	42.8 (37.0–48.6)	0.99 (0.86–1.12)
Setúbal	38.5 (26.6–50.3)	0.97 (0.70–1.26)	46.4 (34.9–58.0)	1.07 (0.80–1.33)
Beja	34.2 (28.1–40.4)	0.86 (0.72–1.02)	34.3 (28.3–40.2)	0.79 (0.67–0.92)
Faro	39.8 (33.9–45.8)	1.01 (0.86–1.16)	41.8 (36.1–47.5)	0.97 (0.84–1.09)

Prevalence considering a 53.2-year mean age and a 42.2% proportion of men in the sample.
Reference class for prevalence ratio estimation:" Deviation coding" – comparison of the individual districts with its global geometric mean.
The PORMETS study was conducted in mainland Portugal from February 2007 to July 2009.

and in the obesity prevalence by 7.4% and 1.3% among women and men, respectively [10]. In addition, diabetes is rising globally [23], and Portugal has one of the highest prevalence rates [12] compared with other European countries [24].

Our study showed a higher prevalence of the "low HDL-C" component than that reported in previous MetS prevalence Portuguese studies [13, 25]. Nevertheless, a recent systematic review that summarizes the evidence from Portuguese studies [26] showed mean HDL-C values discretely higher than ours, for both sexes.

According to our data, the prevalence of the "high triglycerides" component was discretely lower than that in previous Portuguese studies [13, 25]. However, a systematic review [26] that quantified the distribution of lipid fractions found mean triglycerides levels of 150 mg/dL and 111 mg/dL in men and women respectively, similar to our results.

The presence of MetS increases the risk of developing CVD [1] and type 2 diabetes [2]. This study showed that subjects with MetS significantly reported a higher prevalence of previously diagnosed type 2 diabetes, myocardial infarction and stroke, suggesting the association of MetS with CVD and diabetes. However, in Portugal, although the prevalence of MetS is high, the age-adjusted mortality rates from CVD are low, namely when compared

Table 3 Metabolic syndrome prevalence (as defined by HARM2009) by Portuguese NUTS

NUTS II	Prevalence (95%CI)	Prevalence ratio (95%CI)	Prevalence (95%CI)	Prevalence ratio (95%CI)
	Crude		Adjusted for age and sex	
North	40.0 (37.2–42.8)	0.98 (0.90–1.07)	45.0 (41.9–48.1)	0.96 (0.89–1.04)
Center	39.6 (37.1–42.2)	1.00 (0.93–1.07)	43.4 (40.5–46.3)	1.06 (0.99–1.13)
Lisbon	41.7 (37.5–45.8)	0.99 (0.92–1.06)	41.8 (37.7–45.9)	1.02 (0.96–1.08)
Alentejo	39.4 (35.6–43.2)	1.04 (0.95–1.13)	40.8 (40.0–44.6)	0.98 (0.91–1.06)
Algarve	39.8 (33.9–45.8)	0.99 (0.87–1.12)	41.6 (35.9–47.3)	0.96 (0.88–1.03)

Prevalence considering a 53.2-year mean age and a 42.2% proportion of men in the sample.
Reference class for prevalence ratio estimation:" Deviation coding" – comparison of the individual NUTS II with its global geometric mean.
The PORMETS study was conducted in mainland Portugal from February 2007 to July 2009.

Table 4 Demographic, behavioural and analytical characteristics of the Metabolic Syndrome subjects

	With MetS	Without MetS	Prevalence ratio (95%CI) [a]	*p*
Gender Men	656 (39.0)	1026 (61.0)	*	
Women	938 (40.7)	1367 (59.3)	1.15 (1.07–1.23)	< 0.001
Age [years, n (%)] 18–30	21 (5.0)	402 (95.0)	*	
31–40	87 (16.0)	458 (84.0)	3.20 (2.02–5.06)	< 0.001
41–50	222 (30.5)	506 (69.5)	6.09 (3.96–9.37)	< 0.001
51–60	391 (47.3)	435 (52.7)	9.52 (6.23–14.53)	< 0.001
61–70	467 (58.5)	331 (41.5)	11.91 (7.82–18.15)	< 0.001
> 70	406 (60.9)	261 (39.1)	12.40 (8.14–18.90)	<0.001
Education [years, n (%)] 0–4	1054 (53.2)	929 (46.8)	1.11 (0.97–1.28)	0.137
5–12	415 (27.1)	1116 (72.9)	1.03 (0.89–1.20)	0.678
> 12	125 (26.4)	348 (73.6)	*	
Marital status Single/divorced/widower	377 (34.6)	713 (65.4)	*	
Married	1211 (41.9)	1676 (58.1)	0.99 (0.92–1.08)	0.869
Occupation [n (%)] Student	2 (2.4)	81 (97.6)	0.54 (0.14–2.09)	0.372
Unemployed	72 (36.2)	127 (63.8)	1.26 (1.03–1.53)	0.024
Housewife	195 (48.4)	208 (51.6)	1.23 (1.05–1.43)	0.010
Retired	703 (58.4)	500 (41.6)	1.16 (1.00–1.35)	0.046
Blue-collar	251 (29.4)	602 (70.6)	1.03 (0.81–1.20)	0.684
White-collar	193 (24.1)	607 (75.9)	*	
Smoking status [n (%)] Smoker	137 (24.2)	429 (75.8)	0.85 (0.73–0.98)	0.027
Ex-smoker	245 (42.8)	327 (57.2)	1.00 (0.90–1.11)	0.977
Non-smoker	1198 (43.1)	1580 (56.9)	*	
Physical exercise [n (%)] No	1232 (42.9)	1638 (57.1)	1.18 (1.08–1.29)	<0.001
Yes	338 (31.9)	722 (68.1)	*	
Residence [n (%)] Non-urban	835 (43.4)	1087 (56.6)	1.13 (1.05–1.20)	0.001
Urban	759 (36.8)	1306 (63.2)	*	
Weight [kg, Mean (SE)]	78.5 (13.6)	68.1 (12.3)	1.00 (1.00–1.00)	<0.001
Body mass index [kg/m^2, Mean (SE)]	30.0 (4.4)	25.6 (4.0)	1.09 (1.08–1.10)	<0.001
Waist circumference [cm, Mean (SE)]	101.4 (10.1)	88.2 (11.0)	1.00 (1.00–1.00)	<0.001
Hip circumference [cm, Mean (SE)]	108.2 (9.4)	100.5 (8.5)	1.00 (1.00–1.00)	<0.001
Total cholesterol [mg/dL, Mean (SE)]	211.6 (43.0)	207.1 (41.1)	1.04 (0.96–1.13)	0.305
HDL cholesterol [mg/dL, Mean (SE)]	43.2 (11.0)	51.0 (12.5)	0.81 (0.65–1.06)	0.067
Triglycerides [mg/dL, Mean (SE)]	159.0 (86.4)	99.8 (46.3)	1.28 (1.24–1.32)	<0.001
Glucose [mg/dL, Mean (SE)]	105.5 (35.7)	83.1 (16.1)	1.11 (1.07–1.15)	<0.001
Insulin [μU/ml, Median (P25 -P75)]	9.99 (6.80–14.30)	6.50 (4.40–9.50)	1.03 (1.03–1.04)	<0.001
hs-CRP [mg/L, Median (P25 -P75)]	0.22 (0.10–0.47)	0.13 (0.06–0.29)	1.00 (1.00–1.00)	0.107
HOMA –IR [Median (P25 -P75)]	2.43 (1.58–3.72)	1.32 (0.86–1.96)	1.09 (1.07–1.10)	<0.001
Diabetes [n (%)]	451 (76.8)	136 (23.2)	1.65 (1.55–1.75)	< 0.001
Previous myocardial infarction [n (%)]	115 (69.3)	51 (30.7)	1.26 (1.14–1.40)	<0.001
Previous stroke [n (%)]	138 (63.3)	80 (36.7)	1.19 (1.07–1.31)	0.001

MetS: metabolic syndrome (as defined by HARM2009).
The PORMETS study was conducted in mainland Portugal from February 2007 to July 2009.
[a]Prevalence ratio adjusted for sex and age.
*Reference class.

with Western Europe countries [27], and have been decreasing in the last decade [28].

As expected, higher insulin levels and HOMA scores were found in the individuals with MetS. These results are supported by a previous study in the city of Porto [29], stressing the contribution of insulin resistance to the atherogenic profile of the syndrome [30].

According to our data, sedentary behaviour was associated with a higher prevalence of MetS. These results are supported by a recent meta-analysis [31].

Regarding this health problem in our societies, we must consider not only biological but also sociodemographic and psychosocial conditions. Some groups, such as the elderly and the most socioeconomically disadvantaged have a higher risk of MetS [32]. Our data showed an association of MetS with being a housewife, retired or unemployed, corroborating findings from another Portuguese study [33] and from other developed countries [34, 35].

Some differences were found between districts but not by regions of the Portugal mainland (NUTS II). In addition, differences between urban and non-urban populations in the prevalence of the MetS were observed, with this condition being more frequent in non-urban areas. The data from the 1999–2006 National Health and Nutrition Examination Survey also showed that non-urban dwelling was associated with a higher prevalence of MetS among adults in the United States [36]. This disparity, which was also found in other countries [37], may be explained by demographic and socioeconomic factors [38]. However, these factors were addressed in this study and the differences persisted; other explanations must be proposed.

Our study did not address the participants' eating patterns. We cannot exclude the contribution of the food pattern to the differences found in some regions and non-urban areas.

Given the increasing global burden of noncommunicable diseases such as diabetes and CVD, which is driven by forces that include ageing and unhealthy lifestyles, the estimation of the modifiable behavioural, metabolic and physiological risk factors is essential. This study, when documenting a high prevalence of metabolic syndrome in Portugal, with all the risks involved, calls attention to the need for an early diagnosis and therapeutic intervention. The study also draws attention to at-risk groups that deserve special attention. These groups include those living in non-urban areas or districts with a higher prevalence of MetS. The elderly, women, the most disadvantaged socioeconomic categories and individuals with overweight or sedentary lifestyles should also be considered.

Our study includes some strengths, namely the sample size and selection of participants by districts and consideration of urban and non-urban residence. Although our study intends to be representative of the adult Portuguese population, we did not consider individuals not enrolled at health centres belonging to the national health system; however, all citizens have universal access to the national health system. Another limitation of our study was the lack of control of the participants' eating patterns.

Conclusions

This study showed that MetS is highly prevalent in the Portuguese adult population. A high prevalence of hypertension, obesity and diabetes in Portugal, may contribute to these numbers.

Ageing [39] and the trend towards increasing obesity in Portugal [10] are expected to contribute to a future increase in MetS prevalence. Differences in the prevalence of this syndrome were observed by district. In addition, this condition was more frequent in non-urban areas. These results may be useful in selecting priority sites for future national intervention.

Our study provides valuable baseline information for the development of future interventions in Portugal and to assess the trends in the evolution of the MetS and associated risk factors.

Abbreviations

MetS: Metabolic syndrome; CVD: Cardiovascular disease; HDL-C: High-density lipoprotein cholesterol; IDF: International Diabetes Federation; HARM2009: Harmonizing criteria; Wt: Weight; BMI: Body mass index; WC: Waist circumference; HC: Hip circumference; hs-CRP: High sensitivity C-reactive protein; HOMA: Homeostatic model assessment; ATP III: Adult treatment panel III; SD: Standard deviation; PR: Prevalence ratio

Acknowledgements

The authors would like to thank all PORMETS cohort-participants, logistic staff and scientists for their contribution to the study.

Competing interest

The authors report no potential conflict of interest relevant to this article.

Funding

This work was supported by the Insulin Resistance Study Group of the Endocrinology, Diabetes and Metabolism Portuguese Society, Bayer Health Care and Merck Sharp & Dohme Portugal.
ACS holds a FCT Investigator contract IF/01060/2015.

Availability of data and materials

Data sharing is not applicable to this article. However, data and samples collected during the study may become available for collaboration with external researchers once these data and samples have been published and once the doctoral student on the project has completed his thesis.

Authors' contributions

LR, ACS and HB were involved in the conception and design of the study. LR and ACS contributed substantially to the acquisition of data. LR and MS were involved in the statistical analysis. LR, ACS and MS were involved in the analysis and interpretation of data. All authors were involved in drafting the manuscript, approving the final draft, and agree to be accountable for the work. All authors read and approved the final manuscript.

Consent for publication

Not applicable

Ethics approval and consent to participate
All of the Portuguese Regional Health Administrations, the Ethics Committee of the São João Hospital E.P.E. and the Portuguese Data Protection Authority, approved PORMETS. The Clinical Director of each health care centre also provided authorization and all participants provided written informed consent.

Publisher's Note

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Received: 8 January 2017 Accepted: 28 May 2017
Published online: 08 June 2017

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Paper 3: Vitamin D, parathyroid hormone and metabolic syndrome – the PORMETS study.

Raposo L, Martins S, Ferreira D, Guimarães JT, Santos AC.

BMC Endocr Disord 2017;17(1):71. doi: 10.1186/s12902-017-0221-3.

Raposo et al. BMC Endocrine Disorders (2017) 17:71
DOI 10.1186/s12902-017-0221-3

BMC Endocrine Disorders

RESEARCH ARTICLE Open Access

Vitamin D, parathyroid hormone and metabolic syndrome – the PORMETS study

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Abstract

Background: Vitamin D (VitD) and parathyroid hormone (PTH) play important roles in calcium metabolism and skeletal homeostasis. Estimates of the VitD status in several European countries show large variations between them. In addition, no national population-based estimate has been published. VitD and PTH may also play important roles in cardiovascular risk, which has been suggested to be associated with metabolic syndrome (MetS) and is very prevalent in Portugal.

The goal of our study was to evaluate the prevalence of hypovitaminosis D and its determinants as well as PTH serum level determinants and associations of the 25-hydroxyvitamin D and PTH serum levels with MetS and its individual components in a sample of the Portuguese mainland population.

Methods: PORMETS is a national cross-sectional study that includes a total sample of 4095 adults. A subsample, including 500 participants, was randomly selected for the present study. A structured questionnaire was administered to collect information on personal medical histories and socio-demographic and behavioral characteristics. Blood pressure and anthropometrics measurements were performed. Fasting venous samples were collected and PTH and 25-hydroxyvitamin D were measured. VitD adequacy was classified according to the Institute of Medicine, and MetS was classified according to the Joint Interim Statement recommendations. Multiple linear regression and unconditional logistic regression models were used to estimate the associations between the levels of PTH and 25-hydroxyvitamin D and with MetS and its individual components.

Results: The prevalence of VitD deficiency was 37.7%, and MetS was present in 191 participants (38.4%). The serum PTH levels showed a positive association (OR: 1.014; 95%CI: 1.002, 1.026) with the waist circumference component of MetS. The serum 25-hydroxyvitamin D levels were negatively associated with MetS (OR: 0.957; 95%CI: 0.922, 0.993) as well as with its blood pressure (OR: 0.949; 95%CI: 0.912, 0.987) and triglycerides (OR: 0.930; 95%CI: 0.892, 0.969) components.

Conclusion: This study showed a high national prevalence of hypovitaminosis D. The PTH levels showed a significant positive association with the WC component of MetS, and the VitD levels were negatively associated with the BP and triglycerides components as well as with the MetS.

Keywords: Vitamin D, PTH, Functional hypoparathyroidism, Metabolic syndrome, Cardiovascular risk, Prevalence, Portugal

Background

Vitamin D (VitD) is a fat-soluble vitamin that is involved in the metabolism of calcium and skeletal homeostasis [1]. Cholecalciferol (VitD3), the main source of VitD, is synthesized in the skin from the cholesterol precursor 7-dehydrocholesterol through exposure to ultraviolet (UV) B radiation. VitD from dietary sources and sun exposure is not biologically active and must undergo two hydroxylations in the human body for activation. VitD is first hydroxylated by the liver to form 25-hydroxyvitamin D [25(OH)D], also known as calcidiol, which is then primarily hydroxylated by the kidney to form the physiologically active $1\alpha,25(OH)_2D$, or calcitriol. Calcidiol has low bioactivity but is the main form of VitD in the blood stream and best indicator of VitD status.

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According to a recent review [2], estimates of the VitD status in several European countries showed large variations. No national population-based study has been published, and the only available Portuguese studies involved regional or specific groups using hospital-based recruitment. According to a recent study in Portuguese hospitalized patients, VitD deficiency or inadequacy was present in 60.3% of these patients [3].

An inadequate VitD status may play a significant role in cardiovascular disease (CVD) risk [4], and several observational studies suggest an association between hypovitaminosis D and metabolic syndrome (MetS), which represents a cluster of interrelated risk factors for CVD [5]. In addition, MetS is highly prevalent in Portugal. [6] An evaluation of the potential associations of MetS and its individual components with VitD levels may support and enhance current knowledge of the effects of VitD on CVD risk factors.

Parathyroid hormone (PTH), a hormone with a close regulatory relationship with VitD, has also been associated with CVD events [7]. Findings from population-based cross-sectional studies have suggested a positive association between PTH and MetS among older men [8] and in morbidly obese individuals [9]. The goal of our study was to evaluate the prevalence of hypovitaminosis D and its determinants as well as PTH serum levels determinants and associations of the 25(OH)D and PTH serum levels with MetS and its individual components in a sample of the Portuguese mainland population.

Methods

PORMETS (PORtuguese METabolic Syndrome) is a national cross-sectional study that includes a sample of adults registered in primary health care centers of the Portuguese mainland. Information regarding PORMETS recruitment proceedings and methodology have been previously published by the authors [10]. According to national legislation, all citizens are enrolled in the health center of their zone of residence. In each of the eighteen Portuguese mainland administrative regions (districts), two health care centers were included. One was in the district's capital and the other represented a non-urban area. In each center, participants were randomly selected from the general practitioners' lists, and 120 participants were evaluated under an inclusion criterion of 18 years of age or older. The selected participants went to the health center specifically to participate in the study. A total of 4105 participants were evaluated, and information was collected from February 2007 to July 2009. Ten participants were excluded from data analysis because they were pregnant at the time of the interview. Therefore, 4095 participants remained.

For this particular study, 500 participants (286 women and 214 men) were randomly selected from the initial PORMETS sample. This sub-sample size was calculated considering a margin of error of 5%, confidence level of 95% and response distribution of 50% for the proportion of participants with 25(OH)D levels below 30 ng/mL (75 nmol/L). The comparison between the selected and unselected participants did not show significant differences, except for systolic blood pressure and for insulin serum levels (Table 1). The PORMETS study was approved by the Portuguese Regional Health Administrations, the Ethics Committee of the São João Hospital E.P.E. and the Portuguese Data Protection Authority. Additionally, approval from each Clinical Director of the health care centers was received, and all participants provided written informed consent.

A structured questionnaire was administered to collect information on personal medical histories and socio-demographic and behavioral characteristics. The participants were considered current smokers if they smoked daily or occasionally, former smokers if they had stopped smoking for at least 6 months, and non-smokers if they had never smoked. Regarding alcohol intake, participants were categorized as occasional drinkers if they had less than one drink per day, daily drinkers if they consumed at least one drink per day, former drinkers if had stopped drinking for at least 6 months and non-drinkers if they had never consumed any type of alcoholic beverage. Regular physical exercise was considered when the participant was engaged in some leisure time physical activity on a repeated basis for at least 30 min a week. Participants who were evaluated in the June–November period and December–May period were classified, respectively, as "higher" and "lower" UV radiation exposure.

Anthropometrics measurements were performed, including weight, height (Ht) and waist (WC) and hip (HC) circumferences. Weight was measured to the nearest 0.1 kg using a digital scale, and Ht was measured to the nearest centimeter in the standing position using a wall stadiometer. WC was measured midway between the bottom of the rib cage and iliac crest, and HC was measured as the maximum circumference of the buttocks. The waist-to-height ratio (WHtR) was calculated as the WC divided by the Ht, and the waist-to-hip ratio (WHR) was calculated as the WC divided by the HC. Body mass index (BMI) was calculated as weight in kilograms divided by the square of Ht in meters, and participants were classified according to the World Health Organization criteria [11]: underweight, normal range, pre-obese and obese categories, which are defined as BMI < 18.5 kg/m^2, ≥18.5 to <25 kg/m^2, ≥ 25 to <30 kg/m^2 and ≥ 30 kg/m^2, respectively.

Table 1 Comparison between selected and unselected participants

Variables	Unselected participants	Selected participants	*p* value[a]
Gender (n)			
Women	2069	286	
Men	1526	214	0.881
Age – years [median (P25, P75)]	54 (41, 66)	53 (41, 67)	0.856
Education level – years [median (P25, P75)]	4 (4, 10)	6 (4, 10)	0.252
Alcohol intake (n)			
Non-drinker	1618	229	
Former drinker	124	18	
Occasional drinker	934	128	
Daily drinker	892	123	0.990
Smoking habits (n)			
Non-smoker	2486	358	
Former smoker	521	66	
Smoker	529	70	0.597
Physical exercise (n)			
No	2592	352	
Yes	954	139	0.597
UV exposure (n)			
Lower	1971	283	
Higher	1555	217	0.768
Weight – cm [median (P25, P75)]	71.0 (62.5, 80.5)	71.0 (61.0, 80.0)	0.704
Height – cm [median (P25, P75)]	162.0 (155.5, 169.0)	162.0 (155.0, 169.0)	0.745
BMI – Kg/m^2 [median (P25, P75)]	27.0 (24.0, 30.0)	27.1 (24.1, 29.8)	0.813
WC – cm [median (P25, P75)]	93.4 (85.0, 101.5)	93.5 (86.0, 102.0)	0.399
Systolic BP – mmHg [median (P25, P75)]	130 (116, 145)	131 (119, 147)	0.038
Diastolic BP – mmHg [median (P25, P75)]	79 (70, 86)	80.0 (70, 87)	0.073
Glucose – mg/dL [median (P25, P75)]	85 (77, 97)	85 (77, 97)	0.947
Insulin - μU/mL [median (P25, P75)]	7.6 (5.1, 11.4)	8.0 (5.3, 12.2)	0.040
HOMA – median (P25, P75)	1.6 (1.0, 2.6)	1.7 (1.1, 2.9)	0.119
hs-CRP - mg/L [median (P25, P75)]	0.15 (0.07, 0.36)	0.16 (0.08, 0.39)	0.626
Cholesterol – mg/dL [median (P25, P75)]	206 (179, 233)	204 (180, 233)	0.611
Triglycerides – mg/dL [median (P25, P75)]	106 (78, 147)	104 (76, 143)	0.242
HDL-cholesterol – mg/dL [median (P25, P75)]	47 (39, 55)	47 (39, 55)	0.553
MetS (n)			
No	2086	307	
Yes	1403	191	0.428
BP component (n)			
No	1352	177	
Yes	2172	322	0.212
WC component (n)			
No	1812	253	
Yes	1702	247	0.686

Table 1 Comparison between selected and unselected participants *(Continued)*

Variables	Unselected participants	Selected participants	*p* value[a]
Glycemia component (n)			
No	2655	382	
Yes	822	112	0.635
HDL-cholesterol component (n)			
No	1566	224	
Yes	1935	276	0.977
Triglycerides component (n)			
No	2615	383	
Yes	878	117	0.401

SD standard deviation, *UV* ultraviolet, *BMI* body mass index, *WC* waist circumference, *BP* blood pressure, *HOMA* homeostatic model assessment, *hs-CRP* high sensitivity C-reactive protein, *MetS* metabolic syndrome
[a]Chi-square test/*Fisher's* exact test or Mann-Whitney U test *p* value

Blood pressure (BP) was measured on a single occasion using a standard mercury sphygmomanometer with the cuff on the right upper arm after a 10-min rest. Two BP readings were taken and the mean of the two readings was calculated. If the difference between the two measurements was greater than 5 mmHg for the systolic or the diastolic BP, a third measurement was taken and the mean of the two closest values was registered.

Fasting venous blood samples were collected by trained nurses in each health care center, and the samples were stored at −80 °C.

A chemiluminescent immunoassay using a Liaison automated analyzer (Diasorin Iberia, Madrid, Spain) was used to measure 25(OH)D. Biointact PTH and insulin were determined by an electro-chemiluminescent immunoassay using a Cobas e411 automated analyzer (Roche, Amadora, Lisboa, Portugal). High sensitivity C-reactive protein (hs-CRP) was measured using a particle-enhanced immunonephelometric assay on a BN™II laser nephelometer. (Siemens Healthcare, Amadora, Lisboa, Portugal). All other parameters (glucose, total cholesterol, triglycerides, HDL-cholesterol, calcium, phosphorus, albumin and creatinine) were measured using conventional methods with an Olympus AU5400® automated clinical chemistry analyzer. (Beckman-Coulter®,-Oeiras, Lisboa, Portugal). Insulin resistance was estimated by the Homeostatic Model Assessment (HOMA), from fasting glucose (mmol/L) and insulin (μUI/mL), as the product of the two divided by 22.5.

VitD adequacy was classified according to the Institute of Medicine (IOM) recommended cut-off values for 25(OH)D levels [12]: deficiency below 12 ng/mL (30 nmol/L); inadequacy ≥ 12 and <20 ng/mL (≥ 30 and <50 nmol/L) and sufficiency ≥ 20 ng/mL (≥ 50 nmol/L). Hyper- and hypoparathyroidism were defined as PTH levels above and below the standard laboratory reference range (10–65 pg/mL), respectively, and a "blunted PTH response" was defined as a PTH level within the reference range in the presence of 25(OH)D ≤ 12 ng/mL (30 nmol/L) [13].

MetS was defined according to the Joint Interim Statement [14] and was considered to be present if at least three (any) of the following characteristics were present: fasting glucose ≥100 mg/dL or drug treatment for elevated glucose; systolic BP ≥130 and/or diastolic BP ≥ 85 mmHg or antihypertensive drug treatment in a patient with a history of hypertension; triglycerides ≥150 mg/dL (1.7 mmol/L) or drug treatment for elevated triglycerides; HDL cholesterol <50 mg/dL (1.3 mmol/L) in women and <40 mg/dL (1.0 mmol/L) in men or drug treatment for reduced HDL-cholesterol; WC ≥88 cm in women and ≥102 cm in men ("European" criteria).

Statistical analysis: Quantitative data are described as the median values and corresponding 25th (P25) and 75th (P75) percentiles. Counts and proportions were reported for categorical variables. Proportions were compared using the chi-square test or *Fisher's* exact test when appropriate. Mann-Whitney U test was used to compare differences between two independent groups. Multiple linear regression models, with the PTH and 25(OH) D levels as dependent variables, were used to calculate the regression coefficients and their respective 95% confidence intervals (95%CI) of several independent variables, including gender, age, level of education, drinking and smoking habits, UV radiation exposure, physical exercise, Ht, weight, BMI, WC, HC, WHR, WHtR, systolic and diastolic BP, glucose, triglycerides, HDL-cholesterol, total cholesterol, insulin, HOMA, hs-CRP, albumin, calcium, phosphorus and creatinine. The final model was adjusted for age and sex. Unconditional logistic regression models with MetS, its individual components or a "blunted PTH response" as dependent variables were computed, and the odds ratios (OR) and their respective 95% CI were estimated after adjustments for confounding variables.

Results with a two-tailed p value <0.05 were considered statistically significant. Statistical analysis was performed using SPSS version 22* software.

Results

A total of 500 participants (286 women and 214 men) with a median age (P25, P75)) of 53 (41, 67) years [52 (40, 66) years in women and 56 (45, 68) years in men] were included in the present analysis.

The sample characteristics of the 500 participants are presented in Table 2. The median 25(OH)D level was 13.8 ng/mL, with a maximum value of 43.5 ng/mL. According to the VitD adequacy categories, deficiency was present in 37.7% of participants, inadequacy was identified in 47.9% of participants and sufficiency was determined in 14.4% of participants. A seasonal variation of serum 25(OH)D was observed, with significantly higher median values in June–November period compared to the December–May period (p 0.001). The median serum PTH level was 38.1 pg/mL, and hypo- and hyperparathyroidism were present in 0.8% and 9.4% of the participants, respectively.

A "blunted PTH response" was present in 89.2% of the 185 participants, with serum 25(OH)D levels of less than or equal to 12 ng/mL (Table 3). A "blunted PTH response" was more frequent in men (OR: 4.100; 95%CI: 1.289, 13.036), and the frequency decreased with age (OR: 0.960; 95%CI: 0.930, 0.992). Furthermore, participants with a "blunted PTH response" had significantly higher serum phosphorus levels (OR: 4.590; 95%CI: 1.425, 14.781) and lower hs-CRP levels (OR: 0.493; 95%CI: 0.258, 0.943).

The associations of the PTH and 25(OH)D levels with various socio-demographic, behavioral and clinical characteristics are presented in Table 4.

The serum levels of PTH and 25(OH)D showed no significant association between them ($p = 0.770$).

Table 2 Sample characteristics of the 500 participants

	Total	Women	Men	p value[a]
Age (years) - median (P25, P75)	53 (41, 67)	52 (40, 66)	56 (45, 68)	0.072
25(OH)D (ng/mL) - median (P25, P75)	13.8 (9.7,17.6)	13.8 (9.7, 17.5)	13.7 (9.9, 17.9)	0.681
25(OH)D (ng/mL) / UV exposure - median (P25, P75)				
Low UV	13.4 (9.3, 17.5)	13.4 (9.3, 16.6)	13.2 (9.0, 17.9)	
High UV	14.3 (10.0, 18.3)[b]	14.1 (9.9, 18.6)	14.5 (10.2, 17.6)	0.603
VitD adequacy - n (%)				
Deficiency	181 (37.7)	102 (37.5)	79 (38.0)	
Inadequacy	230 (47.9)	133 (48.9)	97 (46.6)	
Sufficiency	69 (14.4)	37 (13.6)	32 (15.4)	0.821
PTH (pg/mL) - median (P25, P75)	38.1 (29.8, 49.2)	38.6 (29.8, 50.1)	37.8 (30.0, 47.7)	0.375
"Blunted PTH response" – n (%)				
No	20 (10.8)	16 (15.4)	4 (4.9)	
Yes	165 (89.2)	88 (84.6)	77 (95.1)	0.023
PTH status – n (%)				
Hypoparathyroidism	4 (0.8)	0 (0)	4 (1.9)	
Normal status	449 (89.8)	254 (88.8)	195 (91.1)	
Hyperparathyroidism	47 (9.4)	32 (11.2)	15 (7.0)	0.021
BMI classification - n (%)				
Underweight	6 (1.2)	5 (1.7)	1 (0.5)	
Normal range	159 (31.9)	94 (32.9)	65 (30.5)	
Pre-obese	213 (42.7)	113 (39.5)	100 (46.9)	
Obese	121 (24.2)	74 (25.9)	47 (22.1)	0.242
Metabolic syndrome - n (%)				
No	307 (61.6)	169 (59.3)	138 (64.8)	
Yes	191 (38.4)	116 (40.7)	75 (35.2)	0.213

SD standard deviation, *25(OH)D* 25-hydroxyvitamin D, *VitD* vitamin D, *UV* ultraviolet, *low UV* period December–May, *high UV* period June–November, *PTH* parathyroid hormone, *BMI* body mass index

[a]Chi-square test/*Fisher's* exact test or Mann-Whitney U test p value; [b]p value <0.001 for the comparison of the mean levels of 25(OH)D according to meteorological periods

Table 3 "Blunted PTH response" characteristics

	Crude OR (95% CI)	OR (95% CI)[a]
Gender		
Women		
Men	3.500 (1.122,10.917)	4.100 (1.289,13.036)
Age	0.964 (0.933,0.996)	0.960 (0.930,0.992)
Height	1.064 (1.006,11.125)	1.002 (0.925,1.085)
BMI	0.936 (0.868,1.010)	0.960 (0.889,1.037)
Systolic BP	0.981 (0.962,1.000)	0.982 (0.958,1.006)
Calcium	1.138 (0.223,5.825)	1.077 (0.211,5.486)
Phosphorus	3.179 (1.173,8.615)	4.590 (1.425,14.781)
Albumin	1.186 (1.050,1.341)	1.138 (0.998,1.299)
Insulin	0.970 (0.909,1.036)	0.969 (0.906,1.036)
HOMA	1.003 (0.979,1027)	1.003 (0.964,1.044)
hs-CRP	0.472 (0.250, 0.892)	0.493 (0.258,0.943)

Some of the variables without significant associations were excluded from the table: education level, drinking and smoking habits, physical exercise, UV exposure, weight, hip and waist circumferences, WHR, WHtR, diastolic BP, glucose, triglycerides, HDL-cholesterol, total cholesterol, creatinine, and 25(OH)D
OR odds ratio, *CI* confidence interval, *BMI* body mass index, *BP* blood pressure, *HOMA* homeostatic model assessment, *hs-CRP* high sensitivity C-reactive protein
[a]OR adjusted for gender and age

Table 4 Associations of PTH and 25(OH)D with socio-demographic, anthropometric, clinical and analytical characteristics

	PTH		25(OH)D	
	β (95% CI)[b]	*p* value	β (95% CI)[b]	*p* value
Gender				
Women	[a]		[a]	
Men	−3.331 (−6.415, −0.246)	0.034	0.383 (−0.666,1.433)	0.473
Age (years)	0.320 (0.225,0.415)	<0.001	−0.029 (−0.061,0.003)	0.080
UV exposure				
Low UV	[a]		[a]	
High UV	−0.531 (−2.541,3.608)	0.734	0.940 (−0.105,1.985)	0.078
Physical exercise				
No	[a]		[a]	
Yes	−2.346 (−5.799,1.087)	0.180	1.655 (0.484,2.826)	0.006
BMI	0.475 (0.143,0.807)	0.005	−0.150 (−0.262, −0.037)	0.009
WC	0.184 (0.048,0.320)	0.008	−0.089 (−0.085,0.008)	0.101
Glucose	−2.319 (−7.704,3.066)	0.398	−2.051 (−3.903, −0.199)	0.030
Triglycerides	−1.118 (−3.557,1.322)	0.368	−1.322 (−2.139, −0.505)	0.002
Calcium	−2.098 (−7.923,3.727)	0.479	0.298 (−1.690,2.286)	0.768
Phosphorus	−4.689 (−7.567, −1.812)	0.001	0.245 (−0.748,1.238)	0.628
Creatinine	14.553 (5.267,23.838)	0.002	2.367 (−0.789,5.522)	0.141
PTH			−0.004 (−0.034,0.025)	0.770
25(OH)D	−0.040 (−0.311,0.230)	0.770		

Some of the variables without significant associations were excluded from the table: level of education, drinking and smoking habits, height, weight, hip circumference, WHR, WHtR, systolic and diastolic BP, HDL-cholesterol, total cholesterol, albumin, insulin, HOMA and hs-CRP
CI confidence interval, *UV* Ultraviolet, *low UV* period December–May, *high UV* period June–November, *IMC* body mass index, *WC* waist circumference, *PTH* parathyroid hormone serum levels, *25(OH)D* 25-hydroxyvitamin D serum levels
[a]Reference class
[b]β coefficients adjusted for gender and age

The serum PTH levels were significantly lower in men (β: -3.331; 95%CI: -6.415, −0.246) and were positively associated with age (β: 0.320; 95%CI: 0.225, 0.415). In addition, positive associations between PTH and BMI (β: 0.475; 95%CI: 0.143, 0.807), WC (β: 0.184; 95%CI: 0.048, 0.320) and creatinine levels (β: 14.553; 95%CI: 5.267, 23.838) were found. Furthermore, a negative association between PTH and serum phosphorus levels (β: -4.689; 95%CI: -7.567, −1.812) was observed.

The serum 25 (OH)D levels were positively associated with participation in physical exercise (β: 1.655; 95%CI: 0.484, 2.826) and were negatively associated with BMI (β: -0.150; 95%CI: -2.262, −0.037) and serum glucose (β: -2.051; 95%CI: -3.903, −0.199) and triglycerides (β: -1.322; 95%CI: -2.139, −0.505) levels.

MetS was present in 191 participants (38.4%), and its prevalence was slightly higher in women than in men, but without statistical significance (40.7% versus 35,2%, $p = 0.213$). The MetS prevalence significantly increased with age ($p < 0.001$) and with higher HOMA scores ($p < 0.001$) as well as higher insulin ($p < 0.001$) and hs-CRP ($p = 0.027$) levels.

Table 5 shows the associations of PTH and 25(OH)D with MetS and its individual components. The PTH serum levels showed a crude positive association with MetS (OR: 1.016; 95%CI: 1.006, 1.027) and its WC (OR: 1.023; 95%CI: 1.012, 1.034) and BP (OR: 1.022; 95%CI: 1.010, 1.034) features. After adjustments for age and sex (model 1), only the association with the WC feature remained significant. This association remained significant even after further adjustment for 25(OH)D levels (OR: 1.013; 95%CI: 1.001, 1.015). After adjustment for age, sex and BMI (model 2), this association lost statistical significance.

We also found a crude negative association between the serum 25 (OH) D levels and MetS (OR: 0.953; 95%CI: 0.921, 0.985) and its BP (OR: 0.951; 95%CI: 0.920, 0.983) and triglycerides (OR: 0.930; 95%CI: 0.893, 0.969) components. After adjustments for age and sex (model 1), all the three associations remained significant. However, after further adjustment for BMI (model 2), the association remained statistically significant only for the BP and triglycerides components of the MetS [(OR: 0.954; 95%CI: 0.916, 0.993) and (OR: 0.937; 95%CI: 0.898, 0.978), respectively].

Discussion

VitD status

We found a high prevalence of VitD deficiency or inadequacy (85.6%) in this sample of the Portuguese population. The high prevalence is supported by previously published national evidence (VitD deficiency or inadequacy ranging from 60.3% to 92.7%), although specific population groups were recruited in hospital settings

Table 5 Associations of PTH and 25 (OH) D with MetS and its components

	PTH		25(OH)D	
	Model 1: OR (95% CI)	Model 2: OR (95% CI)	Model 1: OR (95% CI)	Model 2: OR (95% CI)
MetS	1.004 (0.993,1015)	0.995 (0.983,1.008)	0.957 (0.922,0.993)	0.967 (0.930,1.007)
WC	1.014 (1.002,1.026)[a]	1.002 (0.986,1.017)	0.991 (0.958,1.026)	1.024 (0.980,1.069)
BP	1.006 (0.992,1.020)	1.001 (0.988,1.016)	0.949 (0.912,0.987)	0.954 (0.916,0.993)
Trig	0.996 (0.983,1.008)	0.991 (0.978,1.004)	0.930 (0.892,0.969)	0.937 (0.898,0.978)
HDL	0.998 (0.988,1.009)	0.995 (0.985,1.006)	0.990 (0.959,1.023)	0.997 (0.965,1.030)
Glu	0.998 (0.985,1.010)	0.994 (0.981,1.007)	0.975 (0.936,1.016)	0.990 (0.949,1.033)

OR odds ratio, *CI* confidence interval, *PTH* parathyroid hormone serum levels, *25(OH)D* 25-hydroxyvitamin D serum levels, *MetS* metabolic syndrome, *WC* waist circumference component, *BP* blood pressure component, *Trig* triglycerides component, *HDL* HDL cholesterol component, *Glu* glucose component
Model 1: OR adjusted for gender and age; Model 2: OR adjusted for gender, age and body mass index
[a]OR (95%CI) after adjustment for gender, age and 25(OH)D serum level: 1.013 (1.001, 1.025)

[3, 15, 16]. In addition, according to a recent study, conducted in the city of Porto [17], which included 198 healthy participants, VitD deficiency or inadequacy was present in 48%. In the winter period, these values reached 74%.

Compared with other European [2] and worldwide [18] populations, the median 25(OH)D levels observed in this study were relatively low despite the favorable latitude. The high prevalence of pre-obesity and obesity may have contributed to these figures. Furthermore, VitD intake in the Portuguese population is relatively low [2].

Food fortification, VitD supplementation, sunlight exposure and UV protection habits may also be contributing factors to the differences observed across various countries. Several European countries have adopted measures at the national level to implement VitD supplementation and food fortification policies, but they are not harmonized across Europe [2]. In addition, dietary reference values for VitD intake have been a subject of debate in some countries. In Portugal, there is no legislation of food fortification and VitD supplementation (700–800 IU/day) in adults is only recommended for elderly populations (> 65 years) and subjects with osteoporosis, osteopenia or those with a major risk for osteoporosis [19].

Functional hypoparathyroidism and the association between VitD and PTH

Despite all of the complex interrelationships, no significant association between PTH and 25(OH)D was observed. This lack of association was previously reported [20] and may be partly explained by a "blunted PTH response" to VitD deficiency [13]. In fact, most participants with Vit D deficiency had a "blunted PTH response" (89.2%). This response cannot be explained by the type of definition of hypovitaminosis D used (IOM) because according to its definition only participants with serum levels of 25 (OH) D lower than 12 ng/mL and with normal serum PTH levels were considered. The "blunted PTH response" may correspond to a protective mechanism of bone mass, through the development of a functional hypoparathyroidism [13]. In addition to parathyroid dysfunction, other factors may contribute to the modification of the PTH response to low VitD levels [21–23], such as age, gender, BMI, kidney dysfunction, smoking, and serum calcium levels. Our results did not show a significant contribution of smoking, BMI and serum levels of calcium and creatinine to the "blunted PTH response", but we found positive associations with male gender and serum phosphorus levels and negative associations with age and serum levels of hs-CRP. A positive association was already described between PTH and hs-CRP serum levels [24].

In addition to the "blunted PTH response", other factors may have contributed to the lack of association between the PTH and 25(OH)D levels. The method of measurement strongly influences the VitD and PTH levels and may modify the relationship between them. Specifically, the chemiluminescent immunoassay may have underestimated the 25(OH)D measurements [25]. Regarding the third generation PTH assay, freezing may have contributed to reduced PTH levels [26]. However, previous studies on 25(OH)D showed great stability under freezing conditions [27]. Moreover, despite being a useful biomarker of VitD supply to target tissues, 25(OH)D may not be a good functional marker of the biologically active form, $1\alpha,25(OH)_2D$, relative to PTH regulation. Finally, the upper reference value of normal serum PTH levels (65 pg/mL) that is usually used may not be appropriate as a cut-off point for the definition of high PTH levels [13, 21].

VitD, PTH and MetS associations

To the best of our knowledge, this is the first national study to evaluate 25(OH)D and PTH levels and their associations with MetS.

According to our data, MetS prevalence was high and increased with age, HOMA score, and insulin and hs-CRP levels (data not shown). These results are supported by previous studies [28].

Our study showed a significant positive association between PTH and age even after adjusting for sex. By contrast, no significant association between 25(OH)D and age was observed. According to a systematic review, PTH levels are positively correlated with age [23]. We also found a significant negative association between serum PTH levels and male gender [29].

Although higher PTH levels have been associated with increased risk of CVD [7], insulin resistance, blood pressure and obesity [8, 9] we did not find a significant association between MetS and PTH levels in the adjusted model. Others have found a positive association between PTH and MetS only among older men [8] and in morbidly obese individuals [9].

We found positive associations of PTH serum levels with BMI, WC and the WC component of MetS. As expected, the association with the WC component of MetS was lost after further adjustment for BMI. The link between PTH levels and increased body fat is supported by the evidence of increased body weight in primary hyperparathyroidism [30] and by the positive association with BMI [31]. This association may be even stronger in visceral adipose tissue [32], explaining the stronger correlation found with WC. PTH may increase adipose mass, especially in the visceral compartment, by increasing the influx of calcium into adipocytes.

The 25 (OH) D levels showed a positive association with participation in physical exercise, which persisted after adjusting for sex and age. Regular outdoor physical activity is associated with higher levels of serum VitD [33], which can be partly explained by higher UV radiation exposure. Unfortunately, in this study, no data were available on the specific type of exercise practiced by the participants in our sample (indoor versus outdoor).

MetS was inversely associated with 25(OH)D levels even after adjusting for age and sex; however, the association was lost after further adjustment for BMI. A recent meta-analysis showed that the prevalence of MetS decreased at higher 25(OH)D concentrations [5].

In addition, the prevalence of hypovitaminosis D is generally increased in adults with CVD, namely in coronary heart disease and heart failure [34], and low 25(OH)D levels are associated with an increased risk of ischemic heart disease, myocardial infarction, and premature death [4]. Furthermore, results from a recent meta-analysis indicate a non-linear decrease in overall mortality as the 25(OH)D levels increase [35]. Low levels of vitamin D have been reported to be associated not only with obesity and insulin resistance but also with glucose intolerance, dyslipidemia, increased renin gene transcription, endothelial dysfunction, proliferation of vascular smooth muscle cells, thrombogenecity and inflammation [5]. However, low levels of vitamin D may be simply secondary to inflammation and other phenomena associated with obesity and insulin resistance.

We found a significant positive association of the 25(OH)D levels with BMI, but not with the WC and WC components of MetS. A bi-directional Mendelian randomization analysis [36] suggested a causal role of obesity for the risk of hypovitaminosis D. However, any causal effect of hypovitaminosis D in obesity is likely to be small. The possible mechanisms for the lower 25(OH) D concentrations in obesity [37] include: lower VitD dietary intake, reduced sunbathing habits due to a decreased willingness to expose the body, sedentary lifestyle and mobility limitations, decreased bioavailability due to sequestration in the adipose tissue and a volumetric dilution effect related to greater body weight.

A negative association of 25(OH)D with glycemia was observed, but not with the glycemic component of MetS. Several longitudinal cohort studies and a recent meta-analysis [38] demonstrated an inverse association between the 25(OH)D levels and increased risk of type 2 diabetes. VitD status can interfere with insulin secretion and insulin resistance. VitD stimulation of β-cell VDRs and local activation of the 1-α-hydroxylase enzyme may contribute to the modulation of pancreatic β-cells calcium influx and insulin synthesis. VitD may also increase insulin sensitivity by increasing insulin receptor expression and by stimulating insulin-induced glucose transport.

A negative association was present between the 25(OH)D levels and triglycerides levels [39] and triglycerides component of MetS. Although the mechanisms are not clear, they may involve metabolism of triglycerides through modulation of the intracellular calcium content of adipocytes and hepatocytes. Hypovitaminosis D-related inflammation and insulin resistance may be other contributing factors.

Although no linear association was found between diastolic or systolic BP and the 25(OH)D levels (data not shown), the 25(OH)D levels showed a significant association with the BP component of MetS. According to the literature, VitD is inversely associated with BP [40], and several mechanisms have been proposed to be related to hypovitaminosis D and hypertension [41], including disruption of the negative endocrine regulation of renin gene expression, secondary hyperparathyroidism, and enhanced vascular tone through direct or indirect dysfunction of the endothelial and vascular smooth muscle cells.

Also, the associations observed between Mets and its individual components and PTH and 25 (OH) D serum levels were generally weak. In fact, this was not surprising as the variables considered in those associations were continuous and therefore the calculated ORs represent the odds of an outcome occurring for an increased unit of PTH or 25(OH)D serum levels.

Lastly, one must acknowledge some additional limitations of this study. Firstly, due to its cross-sectional nature, no causal relation can be inferred from the significant associations observed. Also, and finally, one could expect type I error in the evaluation of the crude associations due to multiple comparisons. Nevertheless, the authors do not expect that this could have occurred in the final model defined for this study, as the number of tested variables was in fact small.

Conclusion

The present study showed a high prevalence of hypovitaminosis D in a sample of the Portuguese population. Compared with other European and worldwide populations, our median level of 25(OH)D (13.8 ng/mL) is relatively low. The prevalence of hypovitaminosis D was higher in participants with higher BMI and sedentary lifestyles.

The PTH levels showed a significant positive association with BMI, WC and the WC component of MetS, suggesting a possible role in the pathophysiology of obesity. The 25(OH)D levels were negatively associated with BMI, glucose and triglycerides levels as well as with MetS and its BP and triglycerides components, indicating that hypovitaminosis D may contribute to the pathophysiology of MetS.

Considering the low levels and inadequate intake of VitD, the frequency of overweight, and potentially insufficient solar exposure in the Portuguese population, it is crucial to develop national policies to increase awareness of the importance of VitD for health and to develop strategies for the identification of vitamin D deficiency, especially in at-risk groups.

Abbreviations

25(OH)D: 25-hydroxyvitamin D; BMI: Body mass index; CI: Confidence interval; CVD: Cardiovascular disease; HC: Hip circumference; HOMA: Homeostatic model assessment; hs-CRP: High sensitivity C-reactive protein; Ht: Height; MetS: Metabolic syndrome; OR: Odds ratio; PTH: Parathyroid hormone; SD: Standard deviation; UV: Ultraviolet; VitD: Vitamin D; VitD3: Cholecalciferol; WC: Waist circumference; WHR: Waist-to-hip ratio; WHtR: Waist-to-height ratio

Acknowledgements

The authors would like to thank all PORMETS cohort-participants, logistic staff and scientists for their contribution to the study.

Funding

This work was supported by the Insulin Resistance Study Group of the Endocrinology, Diabetes and Metabolism Portuguese Society.
Ana Cristina Santos holds a FCT Investigator contract IF/01060/2015.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request, once the study has been published.

Authors' contributions

LR, JTG and ACS were involved in the conception of the study. LR was involved in the statistical analysis. SM and DF were involved in the accomplishment of the laboratory exams. All authors were involved in drafting the manuscript, approving the final draft, and agree to be accountable for the work. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All the Portuguese Regional Health Administrations, the Ethics Committee of the São João Hospital E.P.E. and the Portuguese Data Protection Authority, approved PORMETS. The Clinical Director of each health care center also provided authorization and all participants gave their written informed consent. The Ethics Committee of Centro Hospitalar São João (Porto, Portugal) approved the study in the 27th February 2007 and the authorization from the Portuguese Data Protection Authority is CNDP: 1053/2007.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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Received: 6 July 2017 Accepted: 12 November 2017
Published online: 17 November 2017

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Paper 4: Metabolic Syndrome, Thyroid Function and Autoimmunity – The PORMETS Study.

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Submitted to BioMed Research International

Metabolic Syndrome, Thyroid Function and Autoimmunity – The PORMETS Study.

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Abstract

Background: Metabolic syndrome and thyroid dysfunction are the two most common endocrine disorders. Metabolic syndrome, a cluster of interrelated risk factors for cardiovascular disease, is highly prevalent in the Portuguese population. Thyroid dysfunction, primarily hypothyroidism, and Hashimoto´s thyroiditis have also been associated with increased cardiovascular risk, but there are no studies on their prevalence in Portugal.

The aim of our study was to evaluate the prevalence of thyroid dysfunction and antibody positivity as well as to assess the associations of TSH, thyroid hormones and thyroid antibodies with metabolic syndrome, its components, and other possible determinants in a sample of the Portuguese population.

Methods: PORMETS is a nationwide, Portuguese cross-sectional study comprising a sample of 4095 adults. A total of 486 participants were randomly selected to be included in the present study. A fasting venous sample was collected, and the serum lipid profile, glucose, insulin, hs-CRP, TSH, FT4, FT3 and thyroid antibodies were measured.

Results: The prevalence of hypothyroidism and hyperthyroidism measured in our sample was 4.9% and 2.5%, respectively, with a high proportion of subclinical dysfunction identified. Overall, the prevalence of positivity for the TPOAb and TgAb antibodies was 11.9% and 15.1%, respectively. A positive association was found between FT3 and metabolic syndrome (OR: 2.019; 95% CI: 1.196, 3.410). In addition, TPOAb had a negative association with metabolic syndrome (OR: 0.465; 95% CI: 0.236, 0.917) and its triglycerides component (OR: 0.321; 95% CI: 0.124, 0.836).

Conclusions: The present study found a high prevalence of thyroid dysfunction and autoimmunity, and reinforce the association between autoimmunity and thyroid dysfunction. In addition, our data suggest that thyroid autoimmunity, represented by TPOAb, is negatively associated with metabolic syndrome and its triglycerides component, whereas the FT3 levels may be positively associated with the metabolic syndrome risk.

Keywords

Metabolic syndrome, cardiovascular disease, thyroid antibodies, hypothyroidism, hyperthyroidism, thyroiditis, prevalence, Portugal.

Background

Thyroid dysfunction is a common endocrine disorder that includes hypothyroidism and hyperthyroidism, and it may present both overt and subclinical forms [1].

According to a recent meta-analysis on thyroid dysfunction in Europe [1], the mean prevalence of thyroid dysfunction was 3.82%, with 85.2% of cases displaying subclinical forms. Furthermore, according to the same study, the mean total prevalence of hypothyroidism and hyperthyroidism was 3.05% and 0.75%, respectively. The prevalence of undiagnosed hypothyroidism and hyperthyroidism prevalence was 4.94% and 1.72%, respectively.

Worldwide, the most common cause of thyroid disorder is iodine deficiency, which leads to goiter formation and a hypothyroidism state [2]. By contrast, Hashimoto thyroiditis, also known as chronic autoimmune or chronic lymphocytic thyroiditis, is the most prevalent form of autoimmune thyroid disease and is the leading cause of hypothyroidism in iodine-sufficient areas [2]. Hashimoto thyroiditis is characterized by varying degrees of

lymphocytic infiltration and fibrotic transformation of the thyroid gland; high levels of thyroglobulin antibodies (TgAb) and/or thyroid peroxidase antibodies (TPOAb) are usually present in most patients.

Thyroid hormones have important effects on the cardiovascular system [3] and cardiovascular disease (CVD) risk, and mortality may be increased in hypothyroidism [4, 5]. In addition, thyroid autoimmunity may act as an independent CVD risk factor through the promotion of chronic inflammation [6, 7].

Metabolic syndrome (MetS), a cluster of interrelated risk factors for CVD, is highly prevalent in Portugal [8]. According to the literature, thyroid hormones may have a major impact on all the components of MetS, and although the mechanisms remain unclear, the contribution of insulin resistance has been consistently reported [9]. In addition, thyroid hormones have a variety of effects on energy homeostasis, lipid and glucose metabolism and blood pressure [10]. By contrast, the role of thyroid autoimmunity in insulin resistance and in MetS risk is not yet well defined.

The aim of our study was to evaluate the prevalence of thyroid dysfunction and antibody positivity and to assess the associations of TSH, thyroid hormones and thyroid antibodies with metabolic syndrome, its components, and other possible determinants in a sample of the Portuguese population.

Methods

PORMETS (PORtuguese METabolic Syndrome) is a national cross-sectional study that includes a sample of adults registered in primary health care centers of the Portuguese mainland. Information regarding PORMETS recruitment proceedings and methodology have been previously published by the authors [8]. In each of the eighteen mainland administrative regions (districts), two health care centers were included: one was located in the district's capital and the other represented a non-urban area. In each center, 120 participants from the general practitioners' patient lists, aged 18 years or older, were randomly selected. The included participants went to the health center specifically to participate in the study. A total of 4105 participants were evaluated, and information was collected from February 2007 to July 2009. Ten participants were excluded because they were pregnant at the time of the interview. Therefore, 4095 participants remained.

Five hundred participants were randomly selected from the original PORMETS sample. This sub-sample size was calculated considering a margin of error of 5%, a confidence level of 95% and a response distribution of 50% for the proportion of participants with thyroid dysfunction, thyroid antibody positivity or MetS. After the exclusion of 14 participants with missing values for TSH, 486 participants (281 women and 205 men) remained. Participants with previously diagnosed hypothyroidism (n=7) and those under treatment with L-thyroxine were included in the evaluation of the prevalence of thyroid dysfunction but were excluded for the remaining analyzes to avoid the influence of medication on the results.

The comparison between the selected and non-included participants did not show significant differences ($p<0.05$), except for systolic blood pressure (Table 1).

The PORMETS study was approved by all Portuguese Regional Health Administrations, by the Ethics Committee of the São João Hospital E.P.E. (authorized in 27th February 2007), and by the Portuguese Data Protection Authority (authorization number: CNPD 1053/2007). Authorization was also provided by the Coordinator of each health care center, and all study participants gave their written informed consent.

A structured questionnaire was administered by trained nurses to collect information regarding past medical history and socio-demographic and behavioral characteristics, such as education level, smoking and drinking habits and physical exercise.

Anthropometrics measurements were obtained, namely weight, height and waist circumference (WC). Body weight was measured to the nearest 0.1 kg using a digital scale, and height was measured to the nearest centimeter in the standing position

using a wall stadiometer. WC was measured midway between the lower limit of the rib cage and the iliac crest, and body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.

Blood pressure (BP) was measured on a single occasion using a standard mercury sphygmomanometer with the cuff on the right upper arm after a 10-minute rest. Two BP readings were taken, and the mean of the two readings was calculated. If the difference between the two measurements was larger than 5 mmHg for systolic or diastolic BP, a third measurement was taken, and the mean of the two closest values was registered.

A fasting venous blood sample was collected by trained nurses in each health care center, and the samples were frozen and stored at -80º C.

Insulin was determined by an electro-chemiluminescent immunoassay using a Cobas e411 automated analyzer (Roche, Amadora, Lisboa, Portugal). High-sensitivity C-reactive protein (hs-CRP) was measured using the particle-enhanced immunonephelometric assay on a BN®II laser nephelometer (Siemens Healthcare, Amadora, Lisboa, Portugal). All other parameters [glucose, total cholesterol, triglycerides, HDL (high-density lipoprotein)-cholesterol, thyroid stimulating hormone (TSH), free triiodothyronine (FT3), free thyroxine (FT4), TPOAb and TgAb] were measured using conventional methods with an Olympus AU5400® automated clinical chemistry analyzer (Beckman-Coulter®,Oeiras, Lisboa, Portugal).

Insulin resistance was estimated by an equation provided by the Homeostatic Model Assessment (HOMA-IR), from fasting glucose (mmol/L) and insulin (µUI/mL), as the product of the two divided by 22.5 [11]. MetS was defined according to the Joint Interim Statement [12]. MetS was considered present if at least (any) three of the following characteristics were present: fasting serum glucose ≥ 100 mg/dL (6.1 mmol/L) or drug treatment of elevated glucose; systolic BP ≥ 130 mmHg and/or diastolic BP ≥ 85 mmHg or antihypertensive drug treatment in a patient with a history of hypertension; triglycerides ≥ 150 mg/dL (1.7 mmol/L) or drug treatment for elevated triglycerides; HDL- cholesterol < 50 mg/dL (1.3 mmol/L) in women and <40 mg/dL (1.0 mmol/L) in men or drug treatment for reduced HDL-cholesterol; and WC ≥ 88 cm in women and ≥ 102 cm in men (European criteria).

Euthyroidism was defined by normal TSH (0.4 to 3.99 mU/L), FT4 (0.70 to 1.48 ng/dL) and FT3 (1.71 to 3.71 pg/mL) serum levels. Overt primary hypothyroidism was defined by a serum TSH level ≥ 4 mU/L and FT4 serum levels below the lower range. Subclinical hypothyroidism (SCH) was defined as a state of increased serum TSH levels, with circulating thyroid hormone concentrations within the reference range. SCH was divided into two categories according to TSH levels: mildly increased TSH (4.0–10.0 mU/L) and severely increased TSH (>10 mU/L) [13]. Overt primary hyperthyroidism was defined as serum TSH <0.4 mU/L and serum FT4 and/or FT3 levels above the normal range. Subclinical hyperthyroidism (SHyper) was defined biochemically as serum TSH levels below the reference range, with normal thyroid hormone levels. According to its severity, SHyper was divided into two categories [14]: grade 1, which has low but detectable serum TSH levels (0.1–0.39 mU/l), and grade 2, which has undetectable serum TSH levels (<0.1 mIU/l).

The presence of thyroid autoimmune disease was defined by the positivity of any of the two measured thyroid antibodies. Positivity for TPOAb and TgAb was set to values greater than or equal to 5.61 and 4.11 IU/mL, respectively.

Statistical analysis:

Data were described as the mean values and standard deviations (SD). Total counts and proportions were reported for the categorical variables. Proportions were compared using the Chi-square test or *Fisher's* exact test, whenever appropriate. The Student´s t-test was used to compare means of continuous variables.

Multiple linear regression models were used, with thyroid antibodies positivity and TSH, FT4 and FT3 levels as independent variables, and the regression coefficients and their respective 95% confidence intervals (95% CI) were calculated for several dependent variables after adjustment for sex and age. Unconditional logistic regression models were applied, with thyroid antibody positivity, and TSH, FT4 and FT3 levels as independent variable, and odds ratios (ORs) and their respective 95% CIs calculated for several dependent variables after adjustment for sex and age. Dependent variables tested included age, sex, level of education, drinking and smoking habits, physical exercise, WC, BMI, systolic and diastolic BP, glucose, triglycerides, HDL-cholesterol, total cholesterol, insulin, HOMA-IR, hs-CRP, TSH, FT4, FT3, TPOAb, and TgAb levels. MetS and its five components were also tested in these models. Results with a two-tailed value of $p<0.05$ were considered statistically significant. The statistical analyses were performed using SPSS version 22®.

Results

A total of 486 participants (281 women and 205 men), with a mean age (SD) of 53.5 (16.2) years [52.4 (16.3) years in women and 54.9 (16.0) years in men], were included in the present analysis. The mean (SD) TSH, FT4 and FT3 concentrations measured within the study population were 1.70 (2.13) mU/L, 0.94 (0.19) ng/dL and 2.88 (0.43) pg/mL, respectively; men presented with significantly higher FT3 levels compared to women (p=0.019).

The prevalence of previously diagnosed and undiagnosed thyroid dysfunction in this population-based study was 2.1% and 5.3%, respectively (Table 2). The prevalence of hypothyroidism and hyperthyroidism, including subclinical forms, was 4.9% and 2.5%, respectively. According to our results, 88.5% of the undiagnosed thyroid dysfunction cases were subclinical. Thyroid dysfunction was significantly more frequent in women (p=0.012). In addition, the prevalence of hypothyroidism was higher in participants over 50 years of age (p=0.027).

The diagnosis of autoimmune thyroid disease was made in 18.9% of the participants. The overall prevalence of positivity for TPOAb or TgAb was 11.9% and 15.1%, respectively. In addition, both antibodies were positive in 8.0% of the study sample. Positivity for TPOAb or TgAb was approximately 3 times higher in women (p<0.001). In addition, the participants with thyroid dysfunction presented a higher positivity for TPOAb (p<0.001) and TgAb (p=0.021) than the euthyroid subjects. The prevalence of TPOAb and TgAb positivity by type of thyroid dysfunction is presented in Table 2.

MetS was present in 37.8% of the participants (Table 1). The prevalence was 40.7% in women and 33.8% in men (p=0.123). After adjustment for sex and age, and regarding TSH levels, we were unable to identify any association with MetS and its components or with HOMA-IR and insulin and hs-CRP serum levels (Table 3). However, TSH levels were positively associated with physical exercise (OR:1.183; 95% CI:1.020, 1.371), TgAb positivity (OR: 1.295; 95% CI: 1.085, 1.546) and TPOAb positivity (OR: 1.161; 95% CI: 1.015, 1.327). By contrast, TSH levels (Table 4) were negatively associated with FT4 levels (β: -009; 95% CI: -0.017, -0.002).

Serum FT4 levels (Table 3) also showed no association with MetS and its components or with HOMA-IR and insulin or hs-CRP serum levels. However, FT4 levels (Table 4) were positively associated with FT3 (β: 0.034; 95% CI: 0.005, 0.064) and negatively associated with TSH levels (β: -0.009; 95% CI: -0.017, -0.002).

A positive association was found between FT3 levels (Table 3) and MetS (OR: 2.019; 95% CI: 1.196, 3.410), but not with its individual components. Serum FT3 levels were also positively associated with the male gender (OR: 1.859; 95% CI: 1.176, 2.940). In addition, serum FT3 levels (Table 4) were positively associated with insulin (β: 2.042; 95% CI: 0.630, 3453), triglycerides (β: 19.895; 95% CI: 6.951, 32.839) and FT4 (β:

0.934; 95% CI: 0.005, 0.064), levels, and negatively associated with age (β: -9.147; 95% CI: -12.418, -5.877).

The TPOAb positivity (Table 5) showed a negative association with MetS (OR: 0.465; 95% CI: 0.236, 0.917) and its triglycerides component (OR: 0.321; 95% CI: 0.124, 0.836). By contrast, we did not find any association of TgAb with MetS and its components. In addition, thyroid antibody levels presented a significant positive association between them [(OR: 21.933; 95% CI: 10.968, 43.863) and (OR: 22.128; 95% CI: 11.042, 44.343), respectively, for TPOAb and TgAb positivity] and a negative association with male gender [(OR: 0.294; 95% CI: 0.147, 0.558) and (OR: 0.282; 95% CI: 0.152, 0.524), respectively, for TPOAb and TgAb positivity].

In addition to the association with TSH serum levels (Table 3), thyroid antibodies did not show further significant associations (Table 6). Namely, we did not find any associations with HOMA-IR or with insulin and hs-CRP serum levels.

Discussion

Thyroid dysfunction prevalence

The prevalence of thyroid dysfunction (previously known and undiagnosed) in our study was 7.4%, and subclinical forms prevailed (63.9%). The prevalence of hypothyroidism and hyperthyroidism was 4.9% and 2.5%, respectively, and there was a clear female predominance. Compared to other European studies [1], we found a higher prevalence of thyroid dysfunction. We also found a higher prevalence compared to a United States study [15] that showed a prevalence of hypothyroidism and hyperthyroidism of 4.6% (0.3% overt and 4.3% subclinical) and 1.3% (0.5% overt and 0.7% subclinical), respectively. According to our data, undiagnosed thyroid dysfunction was present in 5.3% of the participants (3.5% in hypothyroidism and 1.9% in hyperthyroidism). The prevalence of undiagnosed hypothyroidism and hyperthyroidism was, respectively, lower and higher compared to European findings [1]. According to a United States study [15], the prevalence of undiagnosed hypothyroidism and hyperthyroidism was 4.1% and 0.4%, respectively.

Although the structured questionnaire administered included questioning about drug treatment, the data were incomplete in many participants. Thus, interference of drugs with action on thyroid function cannot be excluded.

The prevalence of thyroid dysfunction may vary according to dietary iodine intake. Data from Portugal point to a borderline iodine intake, namely in pregnant women and school populations [16]. Borderline iodine deficiency has been related to a higher prevalence of hyperthyroidism [17] and may help explain the high prevalence of hyperthyroidism observed in our study.

The associations of TSH with FT4 and with thyroid antibodies were expected. In addition, the associations between FT4 and FT3 are also in agreement with thyroid physiology.

The effect of physical exercise on TSH levels has previously been described but remains controversial [18]. We also found an association of FT3 with age and gender. The finding of decreasing values of FT3 with ageing is supported by the literature [19]. In addition, higher FT3 levels have already been reported in men [20].

Thyroid antibodies prevalence

The prevalence of autoimmune thyroid disease, given by the positivity for TPOAb or TgAb, was elevated in our sample. Even among participants with normal thyroid function, a high prevalence of thyroid antibody positivity was detected.

Epidemiological studies on thyroid antibody prevalence in Europe [21] are difficult to compare due to different epidemiological and analytical approaches. According to a recent review [22], the prevalence for positive TPOAb in European studies varied from 13.9% to 16.9% in women and between 2.9% and 7.3% in men. In addition, the NHANES III study showed a prevalence of positivity for TPOAb and TgAb of 13.0% (17% in women and 8.7% in men) and 11.5% (15.2% in women and 7.6% in men), respectively [15].

Positive thyroid antibodies showed an expected and significant positive association between them and a negative association with the male gender. The high prevalence of thyroid antibody positivity is well documented in women [23]. Genetics related to the X chromosome, parity, steroid hormones, miRNA expression differences and inflammation through leptin and other adipokines have been identified as possible causes. Thyroid antibodies also showed a positive association with TSH levels, supporting the association of autoimmunity with hypothyroidism.

Associations of thyroid function and autoimmunity with metabolic syndrome, its components, and other possible determinants

To the best of our knowledge, this is the first national study approaching thyroid function and autoimmunity and its associations with MetS in a sample of the mainland Portuguese population. Thyroid dysfunction and MetS are very prevalent in Portugal, and our analysis on the association between them may contribute to clarify some questions regarding the role of thyroid hormones in MetS occurrence [9, 10]. In addition, the influence of thyroid autoimmunity in metabolic syndrome is poorly understood, and our findings may help clarify its role.

We did not identify any association between TSH levels and MetS and its components. Observational studies concerning the association between SCH and MetS have shown contradictory results. In addition, two recent meta-analyses also failed to clarify this problem; one of them found a positive association [24] whereas no association was found in the other study [25]. CVD risk and mortality are increased in SCH [4, 5], namely among subjects younger than 65 years and in those with a TSH concentration greater than or equal to 10 mIU/L. However, normal TSH levels are not associated with an increase in CVD risk or mortality [26].

Similarly to TSH, FT4 showed no association with MetS and its components. The contribution of low normal FT4 levels to the development of CVD remains controversial [3, 27]. However, the association of MetS with low normal FT4 levels has been documented in some epidemiological surveys [28, 29]. Furthermore, no association was observed between FT4 levels and HOMA-IR and insulin levels; however, according to a recent study, low normal FT4 levels were associated with insulin resistance in men [30].

Higher FT3 levels were associated with an increased prevalence of MetS. This positive association has been increasingly reported in the literature [20, 29, 31]. Despiste this association, we did not find any association with the individual components of MetS. However, the positive association with the triglycerides component almost reached statistical significance (p=0.092). In addition, the FT3 serum levels were also positively associated with triglycerides serum levels (p=0.003). One possible explanation for the lack of association with any component of the MetS may be that the sample size is not sufficient to achieve statistical significance. In fact, there is a growing evidence of the association of FT3 blood levels with metabolic syndrome and its triglycerides component in euthyroid subjects [29, 32].

Furthermore, FT3 serum levels presented a positive association with insulin (p=0.005) serum levels. Previous studies have shown a significant positive association of FT3 serum levels with HOMA-IR and insulin serum levels [20, 31]. Although a significant association with HOMA was not found, p-value was close to statistical significance (p=0.085).

In addition, no association of hs-CRP with serum levels of TSH, FT4 or FT3 was found. These results are in agreement with the literature [33] and suggest that there is no direct action of the thyroid function indicators on this inflammatory marker.

According to our results, and considering both thyroid antibodies, only TPOAb positivity showed an inverse significant association with MetS and its triglycerides component. The different behavior of the two thyroid antibodies may eventually be explained on the basis of their immunological differences [34]. Unlike TgAb, TPOAb may have a predictive value for the

development of hypothyroidism [35]. However, the presence of TPOAb does not appear to be correlated with CVD risk in patients with SCH [36]. In addition, the analysis of individual participant data from several studies [37] and a 20-year follow-up of the original Whickham survey [38] showed that the risk of coronary heart disease is likely mediated by thyroid dysfunction, without an independent contribution from thyroid autoimmunity.

Evidence on the link between thyroid autoimmunity and MetS (or its components) is lacking. According to the literature, thyroid autoimmunity is not directly involved in MetS pathogenesis [30]. No significant differences were previously reported in HOMA-IR score, insulin levels and MetS and its components according to thyroid antibody positivity [30], namely in obesity [39] and in postmenopausal women [40].The negative association of TPOAb positivity with the triglyceride component is partially supported by a previous report of low fasting levels of serum triglycerides in autoimmune thyroiditis and other autoimmune diseases [41].In addition, we found no association between hs-CRP and thyroid antibodies, which is in agreement with the limited evidence available [42]. Unlike other autoimmune diseases, the systemic inflammatory repercussions of autoimmune thyroid disease may be minor.

The sample size may have conditioned the statistical significance of some of our results. Future studies, with a larger sample size, may clarify some of the doubts raised.

Finally, and due to the cross-sectional design of our study, a cause-effect relationship cannot be established for the associations described.

Conclusions

Our study showed a high prevalence of thyroid dysfunction. In addition, the prevalence of subclinical and undiagnosed thyroid dysfunction was also high.

Thyroid antibody positivity was high in our sample, namely in individuals with thyroid dysfunction. Even among participants with normal thyroid function, a high prevalence of thyroid antibody positivity was observed, which reinforces the need to be aware of the autoimmune thyroid disease.

In addition, no association was observed between TSH and FT4 and MetS and its individual components. By contrast, FT3 levels presented a positive association with MetS and insulin and triglyceride levels, strengthening the plausible role of this thyroid hormone in CVD risk.

Finally, according to our data, a negative association was found between thyroid autoimmunity, represented by TPOAb, and MetS and its triglycerides component. The negative association with triglycerides needs further investigation.

Abbreviations

TgAb: Thyroglobulin antibodies; TPOAb: Thyroid peroxidase antibodies; CVD: Cardiovascular disease; MetS: Metabolic syndrome; SCH: Subclinical hypothyroidism; SHyper: Subclinical hyperthyroidism; WC: Waist circumference; BMI: Body mass index; BP: Blood pressure; hs-CRP: High-sensitivity C-reactive protein; HDL: High-density lipoprotein; TSH: Thyroid-stimulating hormone; FT4: Free thyroxine; FT3: Free triiodothyronine; HOMA-IR: Homeostatic model assessment-insulin resistance; SD: Standard deviation; OR: Odds ratio.

Ethics approval and consent to participate

All the Portuguese Regional Health Administrations, the Ethics Committee of the São João Hospital E.P.E. and the Portuguese Data Protection Authority approved PORMETS. The coordinator of each health care center also provided authorization, and all participants gave their written informed consent. The Ethics Committee of Centro Hospitalar São João (Porto, Portugal) approved the study in the 27th February 2007 and the authorization from the Portuguese Data Protection Authority is CNDP: 1053/2007.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors received a funding from ""Grupo de Estudo da Insulino-Resistência-Sociedade Portuguesa de Endocrinologia, Diabetes e Metabolismo" to the authors members, "Merck Portugal" to Luís Raposo and "Fundação para a Ciência e a Tecnologia" (IF/01060/2015) to Ana Cristina Santos. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Funding

This study was supported by grants from the Insulin Resistance Study Group of the Portuguese Society of Endocrinology, Diabetes and Metabolism. Luís Raposo was funded by "Merck Portugal" through a donation made to the Thyroid Study Group of the Portuguese Society of Endocrinology, Diabetes and Metabolism. Ana Cristina Santos was funded by a "Fundação para a Ciência e a Tecnologia" investigator contract IF/01060/2015.

Authors' contributions

LR, JTG and ACS were involved in the conception of the study. LR was involved in the statistical analysis. SM and DF were involved in the accomplishment of the laboratory exams. All authors were involved in drafting the manuscript and approving the final draft and agree to be accountable for the work. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank all PORMETS participants, logistic staff and general practitioners and nursing staff of the health centers for their contribution to the study.

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Table 1. Comparison between selected and non-included participants

Variables		Non-included participants	Selected participants	p value
Sex - n (%)	Women	2074 (57.5)	281 (57.8)	
	Men	1535 (42.5)	205 (42.2)	0.883
Age – years [mean (SD)]		53.2 (16.3)	53.5 (16.2)	0.743
Systolic BP – mmHg [mean (SD)]		131.6 (22.2)	133.8 (22.6)	0.047
Diastolic BP – mmHg [mean (SD)]		78.1 (12.2)	79.1 (11.7)	0.093
WC – cm [mean (SD)]		93.5 (12.5)	93.7 (12.3)	0.725
BMI – Kg/m^2 [mean (SD)]		27.4 (4.7)	27.3 (4.7)	0.614
Insulin - μU/mL [mean (SD)]		9.1 (6.6)	9.7 (6.6)	0.067
HOMA-IR – mean (SD)		2.2 (2.0)	2.4 (2.3)	0.067
hs-CRP – mg/L [mean (SD)]		0.338 (0.646)	0.330 (0.544)	0.794
Glucose – mg/dL [mean (SD)]		91.9 (27.8)	92.8 (28.8)	0.517
Cholesterol – mg/dL [mean (SD)]		209.2 (42.3)	207.6 (40.6)	0.438
HDL-C – mg/dL [mean (SD)]		47.8 (12.6)	48.2 (12.3)	0.558
Triglycerides – mg/dL [mean (SD)]		124.2 (72.6)	118.7 (63.7)	0.115
MetS - n (%)	No	2092 (59.7)	301 (62.2)	
	Yes	1411 (40.3)	183 (37.8) [a]	0.298
BP component – n (%)	No	1356 (38.3)	173 (35.7)	
	Yes	2182 (61.7)	312 (64.3)	0.258
WC component - n (%)	No	1818 (51.5)	247 (50.8)	
	Yes	1710 (48.5)	239 (49.2)	0.770
Glycaemia component - n (%)	No	2665 (76.3)	372 (77.5)	
	Yes	826 (23.7)	108 (22.5)	0.574
HDL-C component - n (%)	No	1572 (44.7)	218 (44.9)	
	Yes	1943 (55.3)	268 (55.1)	0.956
Triglycerides component - n (%)	No	2625 (74.9)	373 (76.7)	
	Yes	882 (25.1)	113 (23.3)	0.364

All blood samples were taken in the fasted state and the serum glucose, insulin, hs-CRP, cholesterol, HDL-cholesterol and triglycerides were measured.

SD: Standard deviation; BP: Blood pressure; WC: Waist circumference; BMI: Body mass index; HOMA-IR: Homeostatic model assessment-insulin resistance; hs-CRP: High-sensitivity C-reactive protein; HDL-C: HDL-cholesterol; MetS: Metabolic syndrome.

[a] TheMetS prevalence was 40.7% in women and 33.8% in men (p=0.123).

Table 2. Prevalence of thyroid dysfunction and of positive thyroid antibodies according to the type of dysfunction

Thyroid function	Prevalence n (%)	Men/women n / n	Age mean (SD)	TPOAb(+) n (%)	TgAb(+) n (%)	TPOAb(+) or TgAb (+) n (%)	TPOAb(+) TgAb(+) n (%)
Any hypothyroidism	24 (4.9) [a]	6/18	59.0 (14.8)	10 (41.7)	9 (39.1)	11 (45.8)	8 (33.3)
Previously diagnosed hypothyroidism	7 (1.4)	0/7	59.6 (9.2)	4 (57.1)	3 (50.0)	4 (57.1)	3 (42.9)
Undiagnosed hypothyroidism	17 (3.5)	6/11	58.8 (16.8)	6 (35.3)	6 (35.3)	7 (41.2)	5 (29.4)
Overt hypothyroidism	1 (0.2)	1/0	76.0 (-)	0	1 (100)	1 (100)	0
Subclinical hypothyroidism - severe	3 (0.6)	0/3	52.0 (6.2)	3 (100)	2 (66.7)	3 (100)	2 (66.7)
Subclinical hypothyroidism - mild	13 (2.7)	5/8	59.0 (18.2)	3 (23.1)	3 (23.1)	3 (23.1)	3 (23.1)
Euthyroidism	450 (92.6)	197/253	53.1 (16.2)	47 (10.4)	63 (14.0)	80 (17.8)	30 (6.7)
Any hyperthyroidism	12 (2.5) [b]	2/10	56.0 (15.8)	1 (8.3)	1 (8.3)	1 (8.3)	1 (8.3)
Previously diagnosed hyperthyroidism	3 (0.6)	0/3	56.7 (26.1)	0	0	0	0
Undiagnosed hyperthyroidism	9 (1.9)	2/7	55.8 (13.1)	1 (11.1)	1 (11.1)	1 (11.1)	1(11.1)
Overt hyperthyroidism	2 (0.4)	1/1	55.0 (12.7)	1 (50)	1 (50)	1 (50)	1 (50)
Subclinical hyperthyroidism grade 2	3 (0.6)	0/3	54.7 (18.8)	0	0	0	0
Subclinical hyperthyroidism grade 1	4 (0.8)	1/3	57.0 (12.9)	0	0	0	0
Any dysfunction	36 (7.4) [c]	8/28 [d]	58.0 (15.0)	11 (30.6)	10 (28.6)	12 (33.3)	9 (25)
Previously diagnosed thyroid dysfunction	10 (2.1)	0/10	58.7 (14.5)	4 (40)	3 (33.3)	4 (40)	3 (30)
Undiagnosed thyroid dysfunction	26 (5.3)	8/18	57.7 (15.4)	7 (26.9)	7 (26.9)	8 (30.8)	6 (23.1)
Overt dysfunction	3 (0.6)	2/1	62.0 (15.1)	1 (33.3)	2 (66.7)	2 (66.7)	1 (33.3)
Subclinical dysfunction	23 (4.7)	6/17	57.2 (15.7)	6 (26.1)	5 (21.7)	6 (26.1)	5 (21.7)
Total	486 (100)	205/281	53.5 (16.2)	58 (11.9) [e]	73 (15.1) [f]	92 (18.9) [g]	39 (8.0)

Two of the 486 participants included in the analysis had no TgAb assay (one with prior diagnosis of hypothyroidism and one with euthyroidism).SD: Standard deviation; TPOAb: Thyroid peroxidase antibodies; TgAb: Thyroglobulin antibodies.

[a] Prevalence of 2.4% and 6.8% (p = 0.027) respectively below and above 50 years of age; [b] Prevalence of 2.4% and 2.5% (p = 0.948) respectively below and above 50 years of age; [c] Prevalence of 4.8% and 9.3% (p = 0.062) respectively below and above 50 years of age; [d] Thyroid dysfunction was significantly more frequent in women: p=0.012; [e] 16.7% in women and 5.4% in men: p <0.001; [f] 21.5% in women and 6.8% in men: p <0.001; [g] 28.5% in women and 10.2% in men: p <0.001.

Table 3. Associations of TSH and thyroid hormones levels with metabolic syndrome, its components and other categorical variables

Dependent variable	TSH OR (95%CI)	FT4 OR (95%CI)	FT3 OR (95%CI)
Metabolic syndrome	0.935 (0.833, 1.049)	0.503 (0.125, 2.032)	2.019 (1.196, 3.410)*
WC component	0.955 (0.864, 1.056)	0.731 (0.218, 2.450)	1.374 (0.855, 2.207)
BP component	0.955 (0.861, 1.060)	1.631 (0.398, 6.676)	1.203 (0.712, 2.031)
TG component	0.874 (0.712, 1.073)	0.689 (0.188, 2.533)	1.575 (0.928, 2.675)**
HDL-C component	0.989 (0.909, 1.077)	1.060 (0.396, 2.841)	1.099 (0.709, 1.705)
Glycaemia component	0.909 (0.764, 1.081)	0.699 (0.173, 2.814)	1.227 (0.697, 2.159)
Sex (men)	1.011 (0.929, 1.101)	1.040 (0.391, 2.765)	1.859 (1.176, 2.940)*
Physical exercise	1.183 (1.020, 1.371)*	0.900 (0.300, 2.705)	1.285 (0.790, 2.089)
TPOAb positivity	1.161 (1.015, 1.327)*	2.800 (0.887, 8.840)	0.889 (0.458, 1.722)
TgAb positivity	1.295 (1.085, 1.546)*	1.846 (0.553, 6.164)	1.040 (0.575, 1.879)

Logistic regression models adjusted for age and sex. This analysis included 479 subjects. Participants with previously diagnosed hypothyroidism and under treatment with L-thyroxine were excluded (n=7). All dependent variables were treated has dichotomous categorical variables.

TSH: Thyroid stimulating hormone; FT4: Free thyroxine; FT3: Free triiodothyronine; WC: Waist circumference; BP: Blood pressure; TG: Triglycerides; HDL-C: HDL-cholesterol; HOMA-IR: Homeostatic model assessment-insulin resistance; hs-CRP: High-sensitivity C-reactive protein; TPOAb: Thyroid peroxidase antibodies; TgAb: Thyroglobulin antibodies.

* p<0.05; ** p=.0.092.

Table 4. Associations of TSH and thyroid hormones levels with cardiovascular markers and other continuous variables

Dependent variable	TSH β (95%CI)	FT4 β (95%CI)	FT3 β (95%CI)
Age (years)	0.516 (-0.164, 1.196)	4.840 (-3.034, 12.713)	-9.147 (-12.418, -5.877)*
WC (cm)	-0.157 (-0.628, 0.314)	-1.721 (-7.175, 3.732)	0.969 (-1.430, 3.368)
Systolic BP (mmHg)	0.508 (-0.274, 1.290)	1.483 (-7.585, 10.550)	1.029 (-2.959, 5.017)
Diastolic BP (mmHg)	0.142 (-0.332, 0.616)	-3.124 (-8.591, 2.342)	0.306 (-2.109, 2.721)
Triglycerides (mg/dL)	-1.788 (-4.349, 0.772)	-8.104 (-37.830, 21.622)	19.895 (6.951, 32.839)*
HDL-C (mg/dL)	0.210 (-0.288, 0.708)	-2.763 (-8.526, 2.999)	0.778 (-1.758, 3.314)
Glucose (mg/dL)	-0.937 (-2.112, 0.238)	0.177 (-13.550, 13.905)	0.080 (-5.939, 6.100)
HOMA	-0.065 (-0.160, 0.030)	-0.004 (-1.111, 1-103)	0.425 (-0.059, 0.908)**
Insulin (µU/mL)	-0.163 (-0.442, 0.117)	0.165 (-3.078, 3.408)	2.042 (0.630, 3.453)*
hs-CRP (mg/L)	0.001 (-0.022, 0.023)	0.155 (-0.102, 0.411)	0.058 (-0.055, 0.171)
TSH (mU/mL)		-1.252 (-2.289, -0.214)*	-0.060 (-0.519, 0.399)
FT4 (ng/dL)	-0.009 (-0.017, -0.002)*		0.034 (0.005, 0.064)*
FT3 (pg/mL)	-0.002 (-0.020, 0.015)	0.320 (0.045, 0.595)*	

Linear regression models adjusted for age and sex. This analysis included 479 subjects. Participants with previously diagnosed hypothyroidism and under treatment with L-thyroxine were excluded (n=7). All dependent variables were treated as continuous variables.

TSH: Thyroid stimulating hormone; FT4: Free thyroxine; FT3: Free triiodothyronine; WC: Waist circumference; BP: Blood pressure; HDL-C: HDL-cholesterol; HOMA-IR: Homeostatic model assessment-insulin resistance; hs-CRP: High-sensitivity C-reactive protein.

* p<0.05; ** p=0.085.

Table 5. Associations of thyroid antibodies positivity with metabolic syndrome, its components and other categorical variables

Dependent variable	TPOAb (+) OR (95%CI)	TgAb (+) OR (95%CI)
Metabolic syndrome	0.465 (0.236, 0.917)*	0.841 (0.474, 1.493)
WC component	0.984 (0.528, 1.836)	0.893 (0.513, 1.552)
BP component	0.788 (0.394, 1.575)	1.019 (0.546, 1.904)
TG component	0.321 (0.124, 0.836)*	0.710 (0.361, 1.394)
HDL-C component	0.792 (0.440, 1.425)	1.309 (0.764, 2.243)
Glycaemia component	0.637 (0.288, 1.410)	0.764 (0.377, 1.547)
Sex (men)	0.294 (0.147, 0.558)*	0.282 (0.152, 0.524)*
TPOAb positivity		22.128 (11.042, 44.343)*
TgAb positivity	21.933 (10.968, 43.863)*	

Logistic regression models adjusted for age and sex. This analysis included 479 subjects. Participants with previously diagnosed hypothyroidism and under treatment with L-thyroxine were excluded (n=7). All dependent variables were treated has dichotomous categorical variables.

TPOAb: Thyroid peroxidase antibodies; TgAb: Thyroglobulin antibodies; WC: Waist circumference; BP: Blood pressure; TG: Triglycerides; HDL-C: HDL-cholesterol.

* p<0.05.

Table 6. Associations of thyroid antibodies positivity with cardiovascular markers and other continuous variables

Dependent variable	TPOAb (+) β (95%CI)	TgAb (+) β (95%CI)
Age (years)	3.202 (-1.458, 7.862)	0.876 (-3.324, 5.076)
WC (cm)	0.546 (-2.682, 3.773)	0.554 (-2.345, 3.452)
Systolic BP (mmHg)	-3.113 (-8.471, 2.245)	0.227 (-4.623, 5.076)
Diastolic BP (mmHg)	-1.459 (-4.707, 1.708)	0.195 (-2.742, 3.132)
Triglycerides (mg/dL)	-0.055 (-0.231, 0.120)	-0.039 (-0.197, 0.119)
HDL-C (mg/dL)	0.009 (-0.025, 0.043)	-0.007 (-0.037, 0.024)
Glucose (mg/dL)	0.003 (-0.077, 0.084)	0.015 (-0.058, 0.088)
HOMA	-0.036 (-0.686, 0.615)	0.267 (-0.321, 0.855)
Insulin (μU/mL)	0.369 (-1.547, 2.285)	0.751 (-0.971,2.473)
hs-CRP (mg/L)	-0.047 (-0.199, 0.105)	0.037 (-0.099, 0.174)

Linear regression models adjusted for age and sex. This analysis included 479 subjects. Participants with previously diagnosed hypothyroidism and under treatment with L-thyroxine were excluded (n=7). All dependent variables were treated has continuous variables.

TPOAb: Thyroid peroxidase antibodies; TgAb: Thyroglobulin antibodies; WC: Waist circumference; BP: Blood pressure; HDL-C: HDL-cholesterol; HOMA-IR: Homeostatic model assessment-insulin resistance; hs-CRP: High-sensitivity C-reactive protein.

V. Overall Discussion

The present thesis intended to study the MetS and its determinants in Portugal. To reach these objectives, we assessed the cutoff points that best estimated the MetS associated risk for CVD and diabetes in a representative sample of the Portuguese population and the prevalence of MetS itself according to the main definitions, using previously estimated cutoff points (Paper 1). Secondly, the prevalence of MetS was assessed according to the most consensual definition (JIS) and according to cut-off points proposed by the previous study ("European"); several possible determinants of the MetS were also evaluated in the same sample of the Portuguese population (Paper 2). At a later stage, we were able to evaluate other possible determinants of MetS, in a subsample, with a focus on the most frequent endocrine pathology and in particular on VitD deficiency (Paper 3) and thyroid dysfunction and autoimmunity (Paper 4).

To meet the first objective, the cut-off points that better diagnosed the cardiometabolic risk for several adiposity measures were estimated.

According to our results, the correlations of elevated cardiometabolic risk, defined by the presence of two or more of the four JIS criteria excluding the WC component [46, 49], with the different adiposity measures considered, were higher for WHtR, WC and BAI than for BMI in both sexes. Similar results were obtained for the AUC. These findings are in agreement with previous studies showing a slight advantage of WC and WHtR over BMI in estimating the risk of type 2 diabetes [28, 29, 30, 31] and CVD [25, 47-55, 582]. The best performance of these AO measures may be related to the greater

association with VAT [46, 583]. VAT may have a stronger impact than total body fat and SAT on IR [38-43, 337], diabetes [336] and CVD [335] risk.

The stronger association of BAI with cardiometabolic risk when compared to BMI is not supported by the majority of the studies on the subject. [46, 62-65]. In fact, BAI was not designed to estimate diabetes and CVD risk [59]As it was initially proposed as an alternative to BMI for the estimation of body fat mass and percentage of fat, being validated in two specific ethnic groups, Mexican and African Americans. These participants were relatively young (mean age of 35 years) and there was a women predominance. Thus, the differences found regarding the association of BAI with cardiometabolic risk may be related in part to the variety of factors such as ethnicity, age, sex and diabetes and CVD frequency among the populations studied.

The analysis of the association of the various adiposity measures with cardiometabolic risk showed some gender differences. WC, WHtR and BAI disclosed a higher correlation and AUC in women than in men, as previously reported [583, 584].

In addition, women presented lower cut-off points for weight, BMI, WC, WHtR and WHR and higher values for HC and BAI, than men. HC primarily reflects lower abdominal fat deposits of the SAT, which are associated with a lower cardiometabolic risk. In fact, this type of deposits has a negative association with the risk of MetS in both men and women [585]. The storage capacity in the SAT may operate up to a certain limit as a protective mechanism for VAT deposition [586, 587.] Given that women generally have higher mean values of HC [588], it may be assumed that the fat storage capacity in the SAT may be higher in women than in men. Since BAI is derived from

HC, it is reasonable to assume that higher cut-offs in women than in men may reflect HC's influence on the index.

The lower BMI cut-off points found in women are difficult to explain. According to the literature, the BMI related risk of diabetes starts early in men. In fact, at the time of diagnosis, men present lower BMI values than women [419, 589, 590]. In addition, the CVD risk is similar, given the BMI, in both sexes [591].

With regard to AO adiposity measures (WC, WHR and WHtR) our findings are in line with the epidemiological data on obesity gender specificities. Several studies showed, that the type 2 diabetes and CVD risk associated with AO measures, namely WC, can increase in women at lower cut-off points [583, 584, 592-596]. In fact, the Nurses' Health Study showed a RR of type 2 diabetes, for the 90th versus the 10th percentile (92 and 67 cm, respectively) of 5.1 (95%CI 2.9, 8.9) [595]. According to a British study on CHD risk in women, a HR of 3.0 (95%CI 1.8, 4.9) was found for WC of 81 cm or higher (versus WC lower than 70 cm) [594]. In addition, according to a study that compared gender WC differences and CHD risk [596], which included 51,529 men of the Health Professionals Follow-up Study and 121,700 women of the Nurses' Health Study cohort, lower WC thresholds for CHD risk were found in women than in men (71 cm and 84 cm, respectively).

These differences have been related to the gender dynamics of the fat deposits distribution [586, 597, 598]. The percentage of body fat is higher in women for the same BMI [599]. Moreover, men generally accumulate more fat in the upper body and women in the lower body [46]. In fact, for a similar total body fat mass, men present higher WC than women; on the other hand women present higher fat mass to a similar WC [78].

Furthermore, for a given WC, both men and women present similar VAT area. Nevertheless, the correlation of WC with VAT may be higher in women than men (r=0.87 versus r=0.77), and the metabolic effects of VAT may be increased in women [600]. VAT may be more sensitive to the anti-lipolytic effects of insulin in women [601, 602]. Thus, for the same degree of IR, lipolysis may be more active in women than in men. In fact, and according to the number of MetS components present, women have less VAT than men for any number of components [585]. In addition, the association of VAT with the risk of MetS may be stronger in women [OR of 2.13 (95%CI 1.59, 2.84)] than in men [OR of 1.38 (95%CI 1.02, 1.85)] [585].

According to our results, WC and WHR cut-off points showed greater sensitivity in both sexes and WHtR also worked well in women. BMI, WHtR and BAI cut-off points showed greater specificity in both sexes, especially in men. Despite the high sensitivity, WHR presented a low specificity; in addition, it presented greater accuracy in men than in women [603].

Overall, BMI, WC, WHtR and BAI were the adiposity measures with higher accuracy in both sexes. Among these measures, WHtR showed a slight advantage over WC in both sexes. On the other hand, the comparison of BMI with WC showed greater WC sensitivity and BMI specificity in both sexes, as well as greater accuracy and negative predictive value (NPV) for WC and a greater negative and positive likelihood ratio (LR) for BMI, in men. The performance of our estimated cut-off points, namely in terms of sensitivity and specificity, compared with other studies that used similar methodologies, revealed some differences [583, 584, 592].

Taking into account that WC is the most used adipose indicator in the different definitions of MetS and that the differences in diagnosis performance of cardiometabolic risk are not relevant compared to WHtR, our results do not support the substitution of WC by WHtR in the definition of MetS. Nevertheless, taking into account the good results of the WHtR, its use can be suggested in benchmarking studies of populations with diverse ethnic characteristics, because this indicator can attenuate the influence of height in the assessment of abdominal adiposity [57, 604].

According to our results, the estimated cut-off points for WC were 89.0 cm in women and 93.5 cm in men. Compared with the cut-off points proposed by the National Institute of Health (88 cm in women and 102 cm in men) [83], obtained in populations of European origin, our estimates are lower in men. In addition, according to the sex-specific WC cut-off points proposed by WHO [80, 92] for the identification of high (80 cm for women and 94 cm for men) and very high (88 cm for women and 102 cm for men) AO related metabolic risk, our thresholds are similar to those set for high risk in men and very high risk in women. This data suggest, that in women, the cardiometabolic risk associated with WC may start with higher values compared to other populations.

The comparison of the estimated national cut-off points with the results of other worldwide studies [94, 95] is not conclusive considering the dispersion of reported cut-off points (65.5 to 101.2 cm for women and 72.5 to 103.0 cm for men). However, similar cut points were found in two Spanish studies [137, 605] (88.5 to 89.5 cm in women and 94.5 cm in men), probably reflecting the geographical proximity and the genetic and epigenetic similarities with the Portuguese population.

According to the cut-off points proposed by the WHO [92] to define the high and very high risk categories for AO that coincide with the “Europid” and “European” cut-off points of the JIS definition, respectively, the prevalence of AO in our sample was 81.0% and 60.7% in women and 62.1% and 32.7% in men. These results were slightly higher than those found in other national studies [97, 99, 108].

Taking into account that the WC cut-off point for the diagnosis of increased cardiometabolic risk in women was very close to 88.0 cm and to avoid over-diagnosis, we considered the use of European cut-off points more appropriate. In fact, according to the “Europid” cut-offs, most women (81%) would have the WC component of MetS. Thus, we propose, according to the current international recommendations, the use of JIS criteria for the definition of MetS in the Portuguese population using "European" cut-off points.

According to our results, men with a WC of 94.0 cm or more, which meet WHO criteria for high risk AO [92] should also receive increased attention.

According to JIS criteria and using the European cut-off points, the age-adjusted prevalence of MetS in this sample was 45.7%, 39.8% and 43.1%, in women, men and both sexes, respectively. The prevalence was significantly higher in women. The high prevalence of MetS found in our sample is supported by other Portuguese studies [132, 134, 135]. Compared to our results (MetS crude prevalence of 32.7%, 40.0% and 45.9%, according to ATP III, JIS and IDF definitions, respectively), lower prevalence of MetS was found in the EPIPorto study [132] (24.0%, 27.6% and 41.9% according to the ATP III, JIS and IDF definitions, respectively), conducted in the city of Porto during 1999-2003, and including a younger population (mean age of 52.5 years) recruited with a

different methodology. On the contrary, the VALSIM study [133, 135], carried out in mainland Portugal and the islands of Madeira and the Azores, between 2006 and 2007, with different selection criteria and older population (mean age of 58.1 years), showed a higher prevalence (28.4%, 69.4% and 65.5%, according to the ATP III, JIS and IDF definitions, respectively). Another national study, the PREVADIAB study conducted from 2008 to 2009, with a different methodological approach, showed a crude prevalence of 41.5% by IDF criteria [134]. The Portuguese prevalence of MetS is higher than in most European countries, according to studies conducted in Northern [150-159], Western [160-172], Southern [136-149] and Eastern [110, 173-190] Europe. The national prevalence of MetS is also higher than in the USA [193-195, 197, 198]. Compared to other world regions, including Asia-Pacific [205-224], Middle East [225-241], Africa [242-251] and America [196-204], Portugal also presents a high prevalence.

Several other national studies [132, 133] have shown a higher prevalence of MetS in women. Although most European studies showed a higher prevalence in men, some studies have also shown a higher prevalence among women [174, 189, 190, 279, 288, 606]. The higher prevalence of WC and HDL cholesterol components of the MetS in women compared with men may help to explain our results. These two components of the MetS are the only ones that present different thresholds according to gender. Regarding the WC component and as mentioned above, it is well documented that cardiometabolic risk may be initiated for lower WC cut-off points in women [583, 584, 592-596]. Nevertheless, the established cut-off points may not be suitable for different populations, as exemplified by our study. Unlike in men where HDL cholesterol levels are stable throughout their life, in women these levels decrease with age. [607, 608]. Even so, women maintain HDL cholesterol levels about 10 mg / dL higher than men for all

age groups. Taking into account that the impact of low HDL cholesterol levels on CVD, may be higher in women [609-612], their thresholds for the HDL cholesterol component have been adapted [613, 614]. Nonetheless, the cut-off points for HDL-cholesterol may vary according to populations [615]. Additionaly, given the high prevalence of AO in women and its influence on HDL levels [616], it is likely that the higher prevalence of MetS in women is mainly due to obesity.

Comparison of MetS prevalence by JIS criteria with earlier MetS definitions showed a lower prevalence according to the ATP III definition and a higher prevalence according to IDF definition. These differences were confirmed by previous national studies [132, 134, 135].

The discrepancy of these results is justified by the differences in the definition of the MetS components. Thus, according to ATP III the threshold for glycemia is higher than in the other definitions and subjects medicated for risk factors are not included. In the case of the IDF definition in addition to the requirement for the AO component, the cut-off points of this component are lower than in all other definitions.

According to our data the prevalence of the BP, AO and glucose components was 64.3%, 51.0% and 24.9%, respectively. Atherogenic dyslipidemia also played an important role in the prevalence of the MetS (29.4% for triglycerides and 56.5% for HDL cholesterol components, respectively).

The higher prevalence of the BP component of the MetS in comparison with the other components of the MetS can be explained by the high national prevalence of hypertension (42.2%) [130, 617, 618], and because the systolic and diastolic thresholds for

the Mets BP component are lower than those used in the definition of hypertension (respectively 140 and 90 mmHg) [619, 620.]

The high prevalence of the WC component of MetS, only surpassed by the BP component, is also explained by the high national prevalence of obesity [99-102, 107]. However, the estimated prevalence of AO (European thresholds) in the PORMETS study was higher than in other Portuguese studies [97, 99, 108]. In two of them, the same WC measurement methodology was used but they were carried out before 2007, in the periods 1999-2003 [108] and 2003-2005 [97]. In addition, another study [99], temporally closer (2008-2009), used another methodology for WC measurement (immediately above the iliac crest).

According to our data the glucose component of MetS had the lowest contribution to the prevalence of the syndrome, despite the tendency of increase due to the inclusion of diabetic individuals in its definition. Moreover, Portugal has one of the highest prevalence of diabetes in Europe [131, 621, 622], and there is a national trend for increased incidence of diabetes [623], accompanying the overall increase [624]. Nevertheless, taking into account that, the prevalence of diabetes and impaired fasting glycaemia (fasting glycaemia ≥ 110 mg / dL), according to the PREVADIAB study [40], was 11.7% and 8.2, respectively, a slightly higher value than the sum of the two could be anticipated for the glucose component.

Our study also showed a higher prevalence of the HDL cholesterol component and a discretely lower prevalence of the triglycerides component, than that reported in previous national MetS prevalence studies [132, 133]. Nevertheless, according to a recent

national systematic review [625] mean HDL cholesterol and triglycerides levels were similar to our results.

The analysis of the estimates of the age and sex-adjusted prevalence ratio of MetS by districts of Portugal mainland showed some differences. “Vila Real” and "Leiria" districts had the highest prevalence of MetS and, on the contrary, "Bragança" and "Beja" districts presented the lowest prevalence. In addition, participants from non-urban areas presented a higher prevalence ratio than participants from urban areas. Several studies have also found differences between populations living in urban areas and those living in non-urban or rural areas. Some of them [257, 258, 262] also showed a higher non-urban or rural prevalence. On the contrary, other studies showed a higher prevalence in urban populations [208, 252, 256].

Demographic, ethnic, socio-economic and lifestyle factors may have contributed to the differences found in the area of residence and between districts [257, 258, 262, 626]. However, these factors were addressed in our study and did not explain the differences observed. We did not evaluate the participants eating patterns, and therefore the contribution of diet cannot be excluded.

Our results support the association of MetS with increased risk for CVD [381] and type 2 diabetes [436], and also reinforce the evidence on the presence of IR in subjects with MetS [627-629]. In addition to the associations found between MetS and serum insulin and HOMA-IR values, a greater prevalence of previously diagnosed type 2 diabetes, myocardial infarction and stroke, was also found in individuals with MetS. Despite the high prevalence of MetS in Portugal, the CVD mortality rates are relatively low when compared with other Western Europe countries [630-632].

Our study showed the contribution of several other factors to the increased prevalence of MetS. A higher prevalence of MetS was also found in the elderly. This association is well documented [606]. Indeed, several national [132, 133] and international [139, 155, 198, 387, 606] studies have shown an increased prevalence of MetS with age.

Sedentary behaviour was associated with a higher prevalence of MetS. These results are also supported by a recent meta-analysis [633]. The role of sedentary lifestyle had already been highlighted in the AACE statement [9] which had it includedt among the risk factors for IRS. In addition to being able to contribute to IR [634] and to the weight gain [635], through the reduction of energy consumption, it is also associated with a reduction of muscle LPL activity [636] and consequent decrease in muscle uptake of triglycerides and serum levels of HDL cholesterol, as well as with increased postprandial lipaemia [637, 638].

In addition, and as expected, an increased prevalence of MetS was found in subjects with high BMI. This positive association with BMI probably indicates the contribution of adipose tissue to MetS, as discussed above.

The prevalence of MetS was higher in housewives, retired or unemployed participants. The EPIPorto study also found a higher prevalence in housewife and unemployed subjects [639]. This association with socioeconomically disadvantaged groups has already been described [639-642].

The contribution of VitD deficiency and thyroid pathology, namely dysfunction and autoimmunity, for the prevalence of MetS has not been studied in Portugal, despite the fact of its likely high frequency and evidence suggesting the possibility that these endocrine entities might play an active role in the etiopathogenesis of MetS.

According to IOM definition, a high prevalence of VitD deficiency and inadequacy (85.6%) was found in our study. Previous national studies also showed high values, ranging from 48% to 92.7% [473-476].

Despite a favourable latitude, the median 25(OH)D levels found in our study (13.8 ng/mL) were relatively low compared with other European populations [470]. Moreover, comparing with other worldwide estimates our values were also relatively low [478].

Several factors may have contributed to the low levels of levels of 25(OH)D found in our study. Although cutaneous synthesis is the main source of vitD, dietary sources also contribute to the body stores of VitD. In addition to VitD's dietary intake being insufficient in Portugal [470], sunlight exposure and UV protection habits may also have contributed to the low 25(OH)D levels found in our study. Nevertheless, our study did not evaluate the habits of sun exposure and its determinants as well as the use of sunscreens. The high prevalence of overweight participants in our sample (42.7 and 24.2% for pre-obesity and obesity, respectively) may also have contributed to our estimates. Food fortification and VitD supplementation health policies related to the prevention of hypovitaminosis D in Europe are not the same in all countries, as some of them, especially in Northern Europe, have adopted measures to implement VitD supplementation and food fortification [470]. In other countries, such as Portugal, the recommendations are scarce. In fact, there is no national legislation on food fortification and VitD supplementation (700-800 IU/day) in adults, it being only recommended for elderly populations (> 65 years) and subjects with osteoporosis, osteopenia or those with a major risk for osteoporosis [643].

VitD and PTH regulate calcium homeostasis and are part of a feedback system in which PTH stimulates VitD and in turn, VitD has a PTH inhibitory effect. Thus, PTH levels are

usually increased in the presence of ViD deficiency. It would, therefore, be expected to find a significant association between 25(OH) D and PTH serum levels; however, no association was found. This lack of association was previously reported [644]. There may be several justifications for explaining these results. The chemiluminescent immunoassay used for the measurement of serum levels of 25(OH)D may have underestimated the estimates [645]. In addition, prior freezing of serum may have led to a decrease in PTH values, as measured by the third generation PTH assay, used in our study [646]. In contrast, 25(OH)D exhibits great stability under freezing conditions [647]. The conjugation of artificially lower PTH and 25(OH)D values may have also contributed to a high "blunted PTH response" [579] in our study. Although 25(OH)D serum levels may be a good marker of VitD supply to target tissues, they may not be equally suitable to represent the biological activity of 1α,25(OH)^{2}D, regarding the regulation of PTH. Furthermore, the cut-off point considered to define the upper reference value of normal serum PTH levels (65 pg/mL) may be inappropriately elevated, from a physiological point of view [579, 648].

According to our data, PTH serum levels presented a significant positive association with age and a negative one with the male gender. These results are supported by previous studies [649, 650]. By contrast, 25(OH)D serum levels showed no association with gender or age.

Serum levels of PTH and 25(OH)D showed significant positive associations with some adiposity measures. Both showed an association with BMI and only PTH had an association with WC. The association of PTH with adiposity is well documented [651], especially in primary hyperparathyroidism [652]. The effect of PTH on the adipose tissue may be mediated by an increase in calcium influx in adipocytes and subsequent increase in adipose mass, especially VAT [653]. Unlike PTH, any role of VitD in the pathogenesis of obesity is probably small. Indeed, and according to a bi-directional Mendelian randomization analysis, obesity may play a causal role in VitD status [654]. The lower serum levels of 25(OH)D in obesity, may be explained by several mechanisms [655], which include: lower VitD dietary intake, reduced sunbathing habits due to a decreased willingness to expose the body, sedentary lifestyle and mobility limitations, decreased bioavailability due to sequestration in the adipose tissue and a volumetric dilution effect related to greater body weight.

The negative association found between 25(OH)D and glucose serum levels is supported by several studies [513, 516]. In fact, serum levels of 25(OH)D have been associated with a lower risk of type 2 diabetes [656]. VitD may have an effect on glycaemia by stimulating insulin secretion and decreasing IR. The presence of pancreatic 1-α-hydroxylase activity and consequent local production of $1,25(OH)^2D$, as well as the presence of VDRs in β-cell, allows the action of VitD in the modulation of β-cells calcium influx and insulin synthesis. IR may also decrease by VitD related increase of insulin receptor expression and insulin-induced glucose transport.

The negative association found between triglycerides and 25(OH)D serum levels was previously reported [513, 516, 657]. VitD may, by regulating the intracellular calcium

content of the adipocyte and the hepatocyte, influence the synthesis of triglycerides. Other possible mechanisms include inflammation and IR induced by hypovitaminosis D.

The positive association found between physical exercise and 25 (OH) D serum levels may be explained by higher UV radiation exposure. In fact, this association was previously reported for regular outdoor physical activity [658]. Nevertheless, the present study did not characterize the physical exercise in terms of indoor versus outdoor practice.

Despite the reported association of PTH with CVD risk [526], no association was found between PTH serum levels and MetS. In fact the positive association with MetS was only described in older men [510], in morbidly obese [530] and primary hyperparathyroidism subjects [531, 532]. However, a positive association was found between PTH serum levels and the WC component of MetS and PTH serum levels. This association of PTH with adipose indicators and with the WC components of MetS is in agreement with previous studies that support an effect of PTH on fat mass [651-653]. As far as it is possible to know, however, this association with the WC component is not described in the literature, except in older men [510].

Despite the reported association of PTH with systolic and diastolic BP [525, 531, 532], we did not find any association of PTH levels with BP values or with the BP component of MetS. Moreover, we did not find any association of PTH with other cardiometabolic risk factors or with their respective MetS components, namely with glycemia and low HDL cholesterol, previously reported [525].

Unlike PTH, serum levels of 25 (OH) D showed a negative association with MetS, losing its significance after adjustment for BMI.

The association of low levels of 25(OH)D with MetS [502, 509 , 517] and CVD [479, 480, 494] is well documented. Furthermore, overall [659] and CVD [660] mortality may increase as levels of 25(OH)D decrease. Despite the reported association of hypovitaminosis D with CVD, it can not be ruled out that this associationis merely the result of obesity and associated IR [509]. In fact, according to our data, the association is lost after adjustment for BMI.

We found no association of the glucose and triglyceride components of MetS with the 25(OH)D serum levels despite the above-mentioned negative linear associations for glucose and triglycerides serum levels, previously described [513, 516].

In contrast, we found an association with the BP component of MetS although there was no linear association with diastolic or systolic BP. The negative association of VitD with hypertension [483], BP [510, 513, 516] and the BP component of MetS [171, 512, 514, 515] has been previously reported and several mechanisms have been proposed to explain the association. Hypovitaminosis D may have a pressor effect through secondary hyperparathyroidism, a decrease in the inhibitory effect of renin gene expression, and an increase in vascular tonus by dysfunction of the endothelial and vascular smooth muscle cells. Finally, no association was found with WC and HDL cholesterol and its specific components of MetS. Although no association with WC was found, BMI was associated with serum levels of 25 (OHD). Regarding WC, several studies have shown an association of serum 25 (OH) D levels [171, 511, 512, 514, 515] with the WC component of MetS. The association found in these studies may be due to differences in the

characteristics of the samples studied, namely regarding the distribution by sex and age, prevalence of obesity, 25(OH)D serum levels distribution and ethnic specificities. The association with the HDL cholesterol component of MetS has been less reported [171, 515], and several studies, in fact, reported no association at all [511, 512, 514].

Thyroid dysfunction and autoimmunity have also been implicated in the etiopathogenesis of MetS, namely through mechanisms related to the action of thyroid hormones and chronic inflammation, respectively.

In contrast to the malignant pathology [661], there is few available data on the prevalence of thyroid dysfunction and autoimmunity in Portugal. Only one study on the subject, involving pregnant and iodine-deficient women, was published [662]. The prevalence of AIT (AATg and/or AATPO positivity) in this study was 10.7% and 8.0% of pregnant women without AIT, had subclinical hypothyroidism in the first trimester.

The impact of thyroid dysfunction and autoimmune thyroiditis can also be assessed indirectly through consumption of health care. According to a report from the Sentinel Medical Network [663], approximately two thousand thyroid pathology medical appointments were performed by 100,000 National Health Service users during the year 2011; 4.9%, 34.0% and 6.2% of these medical appointments were related to hyperthyroidism, hypothyroidism and thyroiditis, respectively.

According to our results, the prevalence of thyroid dysfunction (previously known and undiagnosed) was 7.4%, with a predominance of subclinical forms (63.9%). The prevalence of hypothyroidism and hyperthyroidism was 4.9% and 2.5%, respectively, with a higher prevalence of all forms of dysfunction in women. Comparing to other European studies [537], and with a United States study [664], we found a higher prevalence

of thyroid dysfunction. In addition, the prevalence of undiagnosed hypothyroidism and hyperthyroidism was, respectively, lower and higher compared to European findings [537].

The high prevalence of hyperthyroidism observed in our study may partially be explained by a borderline iodine intake [665], well-documented in Portuguese pregnant women and school populations [666-668]. Although the structured questionnaire administered included questioning about drug treatment, responses were incomplete in many participants. Taking into account the additional information provided by the questionnaire on a prior diagnosis of hypothyroidism, it was possible to fill in any omissions on L-thyroxine treatment. Thus, interference of drugs, other than L-thyroxine, with action on thyroid function cannot be excluded.

According to our data, TPOAb and TgAb were positive in 11.9% (16.7% in women and 5.4% in men) and 15.1% (21.5% in women and 6.8% in men) of the participants, respectively. A high prevalence of thyroid antibody positivity was also found in participants without thyroid dysfunction. These results are similar to those of other European and USA studies. The estimated prevalence reported for TPOAb positivity in several European studies [669] ranged from 13.9% to 16.9% in women and between 2.9% and 7.3% in men. In the NHANES III study, the prevalence of TPOAb and TgAb positivity was 13.0% (17% in women and 8.7% in men) and 11.5% (15.2% in women and 7.6% in men), respectively [664]. The prevalence of TgAb positivity was higher than for TPOAb. In other studies, the contrary was reported [664]. Nevertheless, the comparison between epidemiological studies on the prevalence of thyroid antibodies can be hampered due to differences in epidemiological approaches, distribution by age

and sex, as well as the ethnic characteristics and iodine intake of the studied populations [670]. In addition to the absence of internationally standard assays, the available ones vary greatly in their cut-off points and their sensitivity and specificity to diagnose the positivity of thyroid antibodies.

In a second step, several possible determinants of serum levels of TSH and thyroid hormones as well as positivity for thyroid antibodies were analysed.

Unlike TSH and FT4, FT3 serum level had a negative association with age and gender (women). These associations were previously reported [554, 562, 671].

Both thyroid antibodies presented a positive association with the female gender. However, we did not find an association with age, as reported in other studies [670]. The higher prevalence of thyroid antibodies and autoimmune disorders in women compared to men is well documented [670, 672]. This association has been mainly related to inflammatory mechanisms mediated by leptin and estrogens through the modulation of the immune response. Additionaly, miRNA expression differences between sexes have also been reported. Thyroid antibodies also showed a positive association with TSH levels, supporting the association of autoimmunity with hypothyroidism.

The thyroid hormones have vast metabolic effects, influencing, in particular, the lipid and glucose metabolism, the BP and body weight [545, 565 673]. Regarding the association of TSH and thyroid hormones with the risk factors that integrate MetS, we only found a positive association of FT3 with triglyceride serum levels ($p<0.05$). Previous studies have shown a significant positive association of FT3 levels with triglyceride levels [551, 559, 564]. We also found an association between FT3 and insulin serum levels. However,

no association was found with HOMA score, but the lack of sample power cannot be disregarded. The association of FT3 with IR was previously documented [551, 564].

Finally, the associations of serum levels of TSH, thyroid hormones and thyroid antibodies positivity with MetS and its components were evaluated. No associations of TSH with MetS and its components were found in our study. Although the participants under treatment with thyroid hormone were excluded, our analysis included 29 participants with thyroid dysfunction. For this reason, comparisons with studies conducted in euthyroid populations may be difficult. Although several studies conducted in euthyroid populations have documented the positive association of TSH with MetS others, on the contrary, did not find this association [547, 550, 554, 559, 560, 562]. We also did not find an association between TSH and triglycerides levels [546, 552, 561] and its MetS component [553, 556, 558] as reported in some studies. Although only a minority of studies suggest a positive association of TSH with the glucose component [549, 562], a recent prospective study has shown an increased risk of type 2 diabetes in participants with low or low-normal thyroid function [543]. Furthermore, as in other studies, we did not find a positive association with IR [552, 553, 555, 556, 561]. Several cross-sectional studies [674, 675] that evaluated the association between SCH and MetS have also found contradictory results. Two recent meta-analyses also failed to clarify this problem, albeit one of them found a positive association [674] whereas no association was found in the other [675]. Furthermore, and although CVD risk and mortality may be increased in SCH [541, 542], namely among subjects younger than 65 years and in those with a TSH concentration greater than or equal to 10 mIU/L, normal TSH levels were not associated with an increase in CVD risk or mortality [676].

We also did not found an association of FT4 with MetS and its components. According to literature, the association of FT4 with MetS is poorly studied, with some studies suggesting a negative association [547, 548, 559, 562]. However, these results were not replicated by other studies [554-556, 560, 563]. In addition, and although some studies have suggested a negative association of FT4 levels with IR [547, 548, 552], we did not find in our study any association with HOMA score or insulin levels. Furthermore, the role of FT4 in CVD risk remains controversial [540].

The FT3 serum levels presented a positive association with MetS. This association has been increasingly mentioned in the literature [551, 559, 560, 562, 677]. We did not find any positive association with the BP, glucose, triglycerides and WC components of the MetS or a negative one with the HDL cholesterol component, as previously reported. Although no association was found with triglycerides component of MetS, levels of FT3 were positively associated with triglyceride levels as reported above. In addition, FT3 levels had a positive association with insulin levels. Several mechanisms have been proposed for the association of FT3 with MetS [551]. Leptin produced by the adipocyte can stimulate the expression of the thyrotropin-releasing hormone gene directly in the paraventricular nucleus and indirectly in the arcuate nucleus, leading to hypothalamic-pituitary-thyroid axis stimulation. The adipose tissue may also be able to increase the conversion of T4 into T3 through the type 1 iodothyronine deiodinase activity. Furthermore, the increase in T3 levels may have a protective function in situations of increased adipose mass leading to increased caloric expenditure. In the fasting state, T3 levels are lower and in situations of overfeeding their levels increase. The proposed mechanisms suggest a role of fat mass and overfeeding in increasing

levels of FT3. Nevertheless, we found no association between FT3 levels and BMI or WC

According to our results, TPOAb presented a negative association with MetS. Despite this, we did not find a negative association of TPOAb with HOMA score or with serum insulin levels. In addition, TPOAb also presented a negative association with the triglycerides component of the MetS. In addition, no associations were found for TgAb. The different behaviour of the two thyroid antibodies may eventually be explained on the basis of their immunological differences [539]. Unlike TgAb, TPOAb may have a predictive value for the development of hypothyroidism [678]. Data on a possible association of AIT with MetS is scarce and the limited evidence available does not favour an association, namely in obesity [679] and in postmenopausal women [573]. According to the literature, thyroid autoimmunity is not directly involved in MetS pathogenesis and no significant associations were previously reported between TPOAb and HOMA, glycaemia and insulin levels [680]. The negative association of TPOAb positivity with the triglycerides component of the MetS is partially supported by a study that reported lower values of triglycerides in AIT and other autoimmune diseases [681]. The mechanisms underlying this action on triglycerides are not yet fully understood but will likely not involve IR.

In addition, no association was found of hs-CRP with thyroid antibodies, which is in agreement with the limited evidence available [682]. Unlike other autoimmune diseases, the systemic inflammatory repercussions of autoimmune thyroid disease may be minor. Moreover, and according to literature [683], there was also no association of hs-CRP with serum levels of TSH, FT4 or FT3.

An association has been already suggested of AIT with the risk of CVD [567-570] and inflammation has been proposed as a possible pathogenic mechanism [684-686]. Nevertheless, TPOAb do not appear to be associated with CVD risk in SCH [687]. Adding to that, the analysis of individual participant data from several studies [571] and a 20-year follow-up of the original Whickham survey [572] suggested that the CVD risk associated with autoimmunity is mediated by thyroid dysfunction.

VI. Conclusions

Adiposity cut-off points for cardiovascular disease and diabetes risk in the Portuguese population: The PORMETS study.

According to our data, WC, WHtR and BAI are the adiposity measures that provided the best evaluation of the adiposity component for MetS in the Portuguese population.

Since WC is currently used as the adiposity measure in the definition of MetS and as no relevant differences were observed between this measure and the other adiposity measures evaluatedWHtR ratio, it is likely that no modification to the current MetS definition need to bewas proposed.

The new WC cut-off points proposed for the Portuguese population (89.0 cm in females and 93.5 cm in males) are very similar to the "European" cut-off points in women and the "Europid" values in men.

Use of the "European" cut-off points may be more appropriate in order to prevent over-diagnosis in women.

The prevalence of the metabolic syndrome in Portugal: the PORMETS study

This study showed that MetS is highly prevalent in the Portuguese adult population. A high prevalence of hypertension, obesity and diabetes in Portugal, may contribute to these numbers.

Ageing [398] and the trend towards increasing obesity in Portugal [96] are expected to contribute to a future increase in MetS prevalence.

Differences in the prevalence of this syndrome were observed by district. Additionaly, this condition was more frequent in non-urban areas. These results may be useful in selecting priority sites for future national intervention.

Our study provides valuable baseline information for the development of future interventions in Portugal and to assess the trends in the evolution of the MetS and associated risk factors.

Vitamin D, parathyroid hormone and metabolic syndrome – the PORMETS study

The present study showed a high prevalence of hypovitaminosis D in a sample of the Portuguese population. Compared with to other European and worldwide populations, our median level of 25(OH)D (13.8 ng/mL) is was considered relatively low. The prevalence of hypovitaminosis D was higher in participants with higher BMI and sedentary lifestyles.

The PTH levels showed a significant positive association with BMI, WC and the WC component of MetS, suggesting a possible role in the pathophysiology of obesity. The 25(OH)D levels were negatively associated with BMI, glucose and triglycerides levels as well as with MetS and its BP and triglycerides components, indicating that hypovitaminosis D may contribute to the pathophysiology of MetS.

Considering the low levels and inadequate intake of VitD, the frequency of overweight, and potentially insufficient solar exposure in the Portuguese population, it is crucial to develop national policies to increase awareness of the importance of

VitD for health and to develop strategies for the identification of vitamin D deficiency, especially in at-risk groups.

Metabolic Syndrome, Thyroid Function and Autoimmunity – the PORMETS Study.

Our study showed a high prevalence of thyroid dysfunction. Moreover, the prevalence of subclinical and undiagnosed thyroid dysfunction was also high.

Thyroid antibody positivity was high in our sample, namely in individuals with thyroid dysfunction. Even amongst participants with normal thyroid function, a high prevalence of thyroid antibody positivity was observed.

In addition, no association was observed between TSH and FT4 and the MetS and its individual components. In contrast, FT3 levels presented a positive association with MetS, and insulin and triglycerides levels, strengthening the plausible role of this thyroid hormone in CVD risk.

Finally, according to our data, thyroid autoimmunity did not contribute to an increase in the prevalence of MetS.was not associated with MetS prevalence. In fact, TPOAb levels presented an inverse association with MetS and its triglycerides component.

VII. References

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